

88
VOL. XVI, PARTS II & III

Published 18 December 1931

The British Mycological Society

(Recognosce notum, ignotum inspicere)

TRANSACTIONS

Edited by

J. RAMSBOTTOM, B. F. BARNES and H. WORMALD

Price to non-members, Fifteen Shillings



CAMBRIDGE UNIVERSITY PRESS

LONDON: FETTER LANE, E.C. 4

ALSO

R. K. LEWIS AND CO., LTD., 136, GOWER STREET, LONDON, W.C. 1

CHICAGO: THE UNIVERSITY OF CHICAGO PRESS

(AGENT FOR THE UNITED STATES)

BOMBAY, CALCUTTA, MADRAS: MACMILLAN & CO., LTD.

TOKYO: THE MARUZEN COMPANY LTD.

PRINTED IN GREAT BRITAIN

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CYCLIC SALTATION IN *STEMPHYLIUM*

By MARGARET A. BRETT

(With Plate III and 4 Text-figures)

A *STEMPHYLIUM* isolated in 1928 has been in culture and under constant observation for the past two years.

When first grown from single spores on Czapek's and Richards's media, a dense mass of darkly sporulating mycelium resulted, apparently homogeneous in nature, but too luxuriant in growth to examine microscopically. The spores from it showed considerable variation in size and appearance (Text-fig. 1).

On dilution plates some colonies appeared, characterised by extremely dense sporulation. It was thought that this might indicate the presence of saltants. In an attempt to diminish the occurrence of such saltations (which have been found to be increased in certain fungi by growth on rich media⁽¹⁾), and also to reduce the luxuriance of sporulation so as to make examination of the plates possible, subsequent cultures were made on media of low nutritive value such as Brown's medium, Dox's agar in a modified and diluted form (from which the phosphate constituent was omitted) and 2 per cent. agar.

EXPERIMENTAL WORK ON THE ORIGINAL *STEMPHYLIUM*(a) *Description of growth on dilute media*

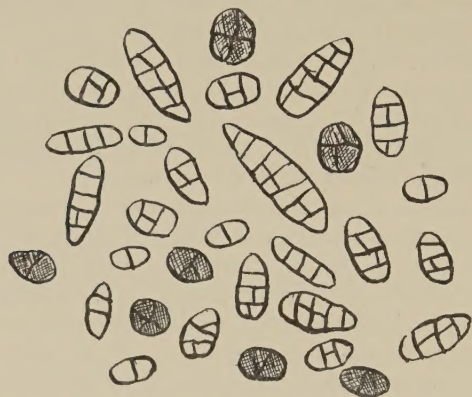
Culturing on dilute media revealed a peculiar growth characteristic of this fungus, which had not before been visible. On Brown's medium, the mycelium apparently spored uniformly for four to six or seven days, then radiating streaks of extremely dense sporulation were formed, like the spokes of a wheel in appearance. These were separated by paler areas, similar to the central region but coloured less deeply. Beyond a diameter of 3.5-4.5 cm., the dark streaks usually gave place to a well marked zone of 0.75-1 cm. in width, brownish in colour, in which sporulation appeared to resemble that in the paler areas. Beyond this, the colony generally began to stale and growth ceased at a diameter of 5.5-6 cm. (Pl. III, fig. 1).

The under side of the plates also showed striking zonation. Over the central area, the medium was stained brownish purple. This colour was very pronounced again in the zone immediately outside the darkly sporulating streaks, while under the dark sectors the medium was always greyish.

A similar habit of growth could be observed on Barnes's medium and less clearly on 2 per cent. agar, since on this medium general

sporulation was much diminished, and the dense radiating streaks were less pronounced. On these media also, the fungus staled before reaching the margin of the plate.

On the diluted and modified form of Dox's medium no staling occurred, and the production of radiating bands of dense sporulation was continued further, generally to a diameter of about 5.5 cm. The cessation of their development was again marked by a definite zone



Text-fig. 1. *Stemphylium* spores (Czapek's agar). $\times 400$.

of about 1 cm. in width, beyond which growth of the fungus continued to the margin of the dish. The medium was more uniformly darkened, but a difference in staining was visible between inner and outer regions. It was considerably darker (almost black) in the central and outer regions, and somewhat paler under the radiating streaks. It may be mentioned also that on Czapek's agar, although no dark radiating streaks were visible owing to general luxuriance of sporulation, a change in the capacity of the fungus for staining the medium was noticed in the middle and outer zones of the plate, the middle zone being paler than the outer. On no plates, however, were the different regions of the mycelium so well contrasted by their different staining properties as on Brown's synthetic medium.

(b) *Microscopic examination on dilute media*

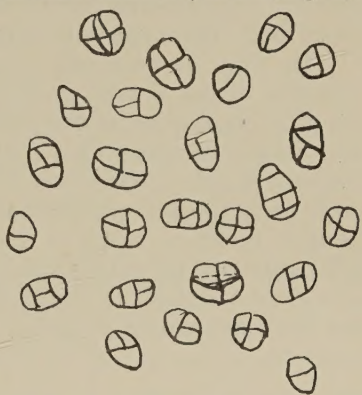
When plates containing the fungus grown on dilute media were examined microscopically, some spores could be seen in the dark radiating bands in extremely dense clusters, each head appearing to be composed of a large number of smaller heads of a densely branching *Stemphylium* type. Many of the spores in these heads bore a smaller spore at the apex. The dense heads also extended feebly into the centre, and on some plates were continued outwards, though more sparsely. They developed chiefly in the dark streaks, but even there,

their appearance was obscured by the spores of the rest of the mycelium. It was only on plain agar plates that they could be seen distinctly, and on these all sporulation was much reduced (Pl. III, fig. 2). The spores composing these heads showed great uniformity in size and shape, averaging $14-18\mu$ by about 12μ (oval) or with a diameter of about 12μ (sarcinaeform). They were very dark-walled when mature (Text-fig. 2).

In the paler areas on Brown's or any other dilute medium, the spores were borne on looser heads of the usual *Stemphylium* type, but there was much greater variation in size and appearance among them, and they were somewhat paler, even when mature. When spore samples were taken from these paler regions, the measurements were found to range from $28-10\mu$ in length, by $17-7\mu$ in breadth. On the various media employed, spore size varied somewhat, but the relative increase of size in spores from the pale areas was always noticed. The above comparative measurements are from a culture on Brown's medium.

Spore samples from the dark areas, however, always included some larger and pale spores. This was due to overgrowth of the mycelium from the pale areas, and was also intensified by the fact that as the cultures aged, aerial hyphae bearing a pale, large, thin-walled and irregular type of spore, developed over the surface of the original mycelium. The amount of this aerial overgrowth varied considerably. In some plates it was abundant, and the hyphae twisted together and often fused, forming a web over the surface of the original mycelium. A curious shrunken and collapsed appearance was presented by these hyphae after spore formation, and the effect was that of a greyish web of secondary infection spreading over the plates.

In the brownish central zone, and in the outer zone of similar appearance, a considerable amount of submerged spore development took place. These spores were small and simpler in form than those on the surface of the mycelium. Submerged spores did not occur in the pale regions between the dark radiating bands nor outside the outer brownish zone, *i.e.* towards the margin of the culture.



Text-fig. 2. *Stemphylium* spores from dark heads (Brown's medium). $\times 400$.

(c) *Behaviour of spores from different regions on subculturing*

When spores were taken from the pale areas of the plates, the resulting colonies resembled those originally obtained and already described. This type will be referred to as the *A* type of colony.

The first inoculations made from the dark bands were from colonies of the *A* type on Brown's medium. This was before microscopic examination of the plates had been made, and before growing the fungus on plain agar. At that time it was thought that saltant colonies might develop from these regions without difficulty, especially as densely sporing saltants had been observed in dilution plates. The results, however, were at first most contradictory, and it was only after repeated trials that colonies of a new type, *B*, were obtained in sufficient numbers to justify the original expectation. The results of six trials are shown in Table I.

Table I

Culture	Spores from dark bands	<i>A</i> type	<i>B</i> type	Non-germinating
1	16	16	—	—
2	20	20	—	—
3	16	8	—	8
4	16	5	9	2
5	20	12	2	6
6	16	4	7	5

It was obvious from these results that spores which would produce the *A* type of colony were also present in the dark bands. Subsequent microscopic examination revealed the fact that the dense dark heads were almost hidden by spores from the overgrowth and adjacent mycelium.

It was later discovered that spores producing the *A* type of colony germinate quickly, and since in the first two cultures selection was made of those with germ tubes visible, the preponderance of the *A* type in the results is explained. In the four following cultures, when spores were selected with or without germ tubes, the result was a proportion of colonies of the type *B*, and a large proportion of spores which failed to germinate.

This led to attempts at very careful selection of spores from the dense heads on agar plates at an early stage before the overgrowth had developed to any extent. Although the germination results were poor, the successful germinations with two exceptions produced the new type of colony. The results are shown in Table II.

Colonies of type *B* had a densely sporulating centre in which only the large heads of spores were present. Many of the spores bore another spore at the apex. Beyond this central black area, extending from 0.75–1.5 cm. on Brown's medium, the type of growth was

similar to that produced by spores from the pale areas, *i.e.* radiating bands of denser sporulation separated by paler regions. After the colony had attained a diameter of about 4 cm. the paler sporulating mycelium generally grew out forming definite sectors in four or five regions (Pl. III, fig. 3, lower colony).

Table II

Culture	Spores from dense heads	Type A	Type B	Non-germinating
1	16	—	8	8
2	16	2	9	5
3	20	—	12	8
4 (old)	20	—	7	13
5 (old)	20	—	5	15

All plates of type *B* were very slow growing at first as compared with type *A*. The central portion of all media used was stained exactly as under the dense bands of spores in type *A*, while the colour characteristic of the paler regions was found under the outgrowing sectors. Submerged spores were clearly visible under the sectors, and there were indications also of the dense heads of spores on some plates which did not stale early.

(d) *Hyphal tip cultures*

Cultures from hyphal tips produced a similar result. When these were taken from the edge of a growing culture which had passed the densely sporulating zone, the resulting colonies were all of type *A*. Attempts were also made to select hyphal tips from very young cultures on densely sporing plates, tracing back the hypha to a dense head of spores. Their behaviour, however, was not consistent and it was found difficult to select them with certainty. Some tips produced a very small central tuft of densely sporulating mycelium, but most grew at once into a colony of the *A* type.

EXPERIMENTAL WORK ON "*ALTERNARIA*" FORMS OF
DENSE SPORULATION

(a) *Description of Alternarias first obtained*

Reference has already been made to the fact that in dilution plates a somewhat different type of colony was observed on several occasions. These were densely sporing, but differed in the fact that the spores were formed in chains of more than two, arising from the dense heads, thus resembling the form-genus *Alternaria* (Text-fig. 3).

Plating from these colonies showed that some spores from them behaved similarly to spores from the dense heads already described. This behaviour in an *Alternaria* is similar to that described by Wiltshire (2).

The history of some of the *Alternaria* spp. has been followed in detail.

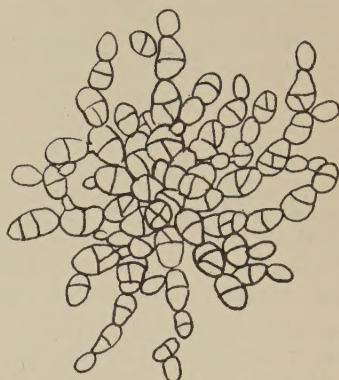
They were chosen in preference to the other more *Stemphylium*-like types of dense sporulation because of the distinctive character of their method of spore production, because they were met with at an early stage in the work, and because some of the results obtained of their behaviour, preceded chronologically those of the first section. More interest was felt in this type also from a systematic point of view.

Several such densely sporing *Alternaria* forms were produced from a single spore culture on agar (L4) which was of the *A* type. When spores were plated out by the dilution method, two plates yielded colonies characterised by even denser sporulation than the usual dark spored type, being visibly different to the naked eye. On one plate, sixteen such colonies occurred out of fifty-two, on the second, two only out of forty-nine.

When single spores from these colonies were grown on various media, out of sixty-three spores, eighteen produced the *A* type of colony, though somewhat darker, and forty-five behaved in the manner described by Wiltshire. (Only those with germ tubes visible were selected.) In these a colony showing the *Alternaria* character first appeared varying in diameter on different media (on Brown's medium it measured from 0.75–1.5 cm.). On some plates the characteristic radiating growth began, the spores in the dark streaks showing the chaining character and the areas in between being occupied by *Stemphylium*. Sectors of *Stemphylium* grew out in several places at the margin of the colony and gradually surrounded the *Alternaria* region. These colonies were distinguishable microscopically from the other dark-spored type *B* only by their darker colour. On other plates the dense central area was larger, up to 2.5 or 3 cm.; the radiating growth was not visible, but sectors of *Stemphylium* grew out, and gradually formed a surrounding zone. In the first type of plate there appeared to be a circular dark area, in the second an irregular star-shaped area of *Alternaria*.

Under the *Alternaria* region the different media employed were always stained as under the densely sporing areas of types *A* and *B* already described, and under the outgrowing sectors as under the paler regions. The *Alternaria* form compares in this respect with the type *B* colonies already described.

Subsequent platings from the central region of the *Alternaria* plates gave two types of colony:—



Text-fig. 3. Surface view of dense head of spores, *Alternaria* forms. $\times 300$.

(a) *Alternaria*, which again formed reverting sectors of *Stemphylium* (cf. type B).

(b) The A type of colony, but darker in colour. Since these differ somewhat from the A and B types already described they will be distinguished as A^1 and B^1 .

From the central region of the B^1 colonies up to a diameter of about 1 cm. only a very occasional type A^1 plate was obtained; most were of the B^1 type, which repeated the behaviour already described.

From the area immediately outside the central region, until the colony attained a diameter of 3.5–4 cm. the chances of picking up *Alternaria* spores which would give the B^1 type, or *Stemphylium*, which would give the A^1 type of colony were about equal. More of the A^1 type actually developed, owing to the greater germination capacity of these spores.

From the outer region only the type A^1 colony was obtained.

On the modified form of Dox's medium used, the *Alternaria* region extended further. Sectors of *Stemphylium* did not usually appear until within about 2 cm. of the margin of the plate, growth of these sectors then continuing to the margin itself (Pl. III, fig. 4).

When plates on which growth was taking place on diluted media were examined under the microscope, hyphae bearing *Stemphylium* spores could be traced almost to the centre of the plate, though those showing the *Alternaria* character predominated at first. The reversion is thus initiated at a point very near to the centre, as in all colonies of the B type which have been examined.

Practically the whole of the central region is thus heterogeneous in character, being a mixed mycelium of the *Alternaria* form and the *Stemphylium*. The *Alternaria* as well as the *Stemphylium* may show some submerged spore development which increases where the *Alternaria* ceases to grow radially. Under the *Alternaria* region, these submerged spores are in chains.

Alternarias which constantly reverted could always be obtained by selecting spores from the centre. Two of these, 10a and 10b have been plated out, at intervals of six weeks during the past year, without any noticeable change of the *Alternaria* character.

On a few plates which were non-staling there was a slight development of dense heads of spores, in the *Stemphylium* sectors, but this was not pronounced. This is readily understood when it is remembered that at the diameter at which *Stemphylium* sectors were formed, the dense heads of spores in the A type of colony were hardly ever visible. A colony of type B is therefore unlikely to arise from spores taken from these *Stemphylium* sectors.

(b) *Recurrence of Alternaria habit in subcultures*

It has been shown that the *Alternaria* form of dense sporulation, once obtained, can be handed on through successive generations by selecting spores from the central area of colonies of type B^1 . It was also found that the character reappeared in subsequent plates derived from spores taken from type A^1 colony.

Single spores from the dark regions of one such plate (in which the production of spores in chains was not obvious), gave 60 per cent. of slow growing colonies characterised by the production of abnormally branched, much swollen submerged hyphae (Pl. III, fig. 4) which gave a brownish streaked appearance to the plates. Above these hyphae the spores were borne in chains. Sectors of *Stemphylium* grew out from one or more portions of the colony, but the *Alternaria* hyphae showed a more extended radial growth than in the original *Alternaria* plates. While the nature of this colony did not differ greatly from those originally obtained, the appearance was strikingly different, owing to the extensive development of the peculiar hyphae already mentioned.

Slow growing colonies of this type appeared on various media, including 2 per cent. agar. Pl. III fig. 6 shows two colonies of the same age (twelve days) on Brown's medium, one of the A^1 type, the other of this slightly different B^1 type.

In the following table the numbers are given of these colonies for seven successive reculturings;

Table III

Plate	Inoculations	Type B^1	Type A^1
m_{12}	16	10	6 (= m_{12a})
m_{12a}	16	8	8 (= m_{12b})
m_{12b}	20	15	(2 infec.) 3 (= m_{12c})
m_{12c}	16	7	9 (= m_{12d})
m_{12d}	16	9	(2 infec.) 5 (= m_{12e})
m_{12e}	16	6	12 (= m_{12f})

Single spores were again taken from the *Alternaria* regions of the B^1 type, forty-eight spores being tested in all. Cultures have also been made from single hyphal tips of the *Alternaria* hyphae, which were readily picked out by reason of their abnormal appearance.

In all cases both from spores and hyphal tips, sectors of *Stemphylium* grew out as in the colonies previously examined. The central portions of the colonies showed both *Alternaria* and *Stemphylium* types of sporulation.

This reappearance of the *Alternaria* character in slow growing colonies, associated with abnormal hyphal development, recalls the description given by Wiltshire, in which he speaks of the *Stemphylium* mycelium being swollen and dark coloured in the *Alternaria* region.

EXPERIMENTAL WORK ON GERMINATION, GROWTH RATE, AND
STALING CAPACITY OF THE TWO TYPES OF SPORES

(For convenience, those from the dense heads will be distinguished as *B* spores and the rest as *A* spores.)

(a) Germination

Spores from the dense heads, whether in chains or otherwise, showed a lower percentage of germination than spores from the more feebly sporulating part of the mycelium. This is shown in the following table.

Table IV

Plate	<i>B</i> spores	Germinated	<i>A</i> spores	Germinated
<i>D</i> I	24	14	24	23
<i>D</i> II	16	8	16	16
<i>D</i> III	24	10	24	22 (2 infected)
<i>D</i> IV	18	10	18	18
<i>D</i> V	16 (<i>Alternaria</i>)	10	16	15
<i>D</i> VI	16 „	12	16	16

These spores were taken from cultures three weeks old. As the colonies age, a smaller percentage of *B* spores seem to be capable of germination, but exact experimental evidence has not yet been obtained on this point.

When germination occurs, the germ tubes from *B* spores are slower in development than those from *A* spores. A preliminary investigation on this point revealed that after eighteen hours the average length of the first germ tube of the *B* spore was 36μ , while that of the *A* spore was 130μ . The age of the colony from which these spores were taken was three weeks, but further evidence is needed to discover if this difference is more pronounced in older cultures.

(b) Growth rate and staling capacity

Colonies obtained from *B* spores were always slower in radial growth than those from *A* spores. This was very pronounced in the *Alternaria* type, as is shown in the following table of measurements.

Table V

	Day	Diameter in cm. of colony Type <i>B</i> ¹	(Average of four taken) Type <i>A</i> ¹
On Brown's medium	2	0.1	0.3
Temp. $22\frac{1}{2}^{\circ}\text{C}$.	3	0.4	1.2
	4	1.1	2.4
	5	1.75	3.3
	6	2.4	3.9
	8	2.6	4.7

After the eighth day, the outgrowing sectors destroyed the symmetry of the type *B*¹ colony.

It was noticed that the growth of type *B*¹ colony slowed down before the appearance of *Stemphylium* sectors. This suggested the idea that possibly staling products had begun to accumulate at the margin of the colony, which were tolerated by the *Stemphylium* but not by the *Alternaria*.

The following preliminary experiments seem to support this view. In six plates of the type *A*¹ colony, the medium (Brown's) was inverted after nine days' growth. The formation of the dense streaks had ceased, but the mycelium was still growing radially.

Four *Alternaria* spores, with the germ tubes already visible, were placed on the inverted medium, one centrally and three at equal distances round the margin of the plate. Of these twenty-four spores, twenty-two failed to continue growth. None of the six centrally placed grew; two of the outer ones continued development, forming small colonies, but these did not show the *Alternaria* character. (These were probably mistakenly selected.)

This experiment was repeated with twenty-four *A* spores taken from *Stemphylium* sectors. Again none of the central spores continued growth, but twelve in the outer regions formed small colonies of the *A* pattern.

The medium in the centre was presumably too full of staling products to permit of growth of either kind of spore. Growth of the *Alternaria* spore cannot continue in a medium partly staled by the *Stemphylium*, but the *Stemphylium* spore itself is less sensitive to the presence of these staling products.

Since the production of staling substances is intimately related to the composition of the medium, it would appear to be possible to adjust a synthetic medium so that growth of the *Alternaria* could be extended, possibly to such an extent that it would occupy the margin of the plate instead of the *Stemphylium*. Experiments are now in progress to determine the effect of varying the Carbon-Nitrogen ratio, the concentration of the constituents, and the acidity of the medium on the relative growths of *Alternaria* and *Stemphylium* and on their staling reactions.

Closely connected with the slow rate of germination and growth of the *Alternaria* spores is the result when a spore sample containing mixed spores is used as inoculum instead of a single spore or hyphal tip. The *Alternaria* character never asserts itself, and the colony is of the *A* type. The more dominant *A* spores grow vigorously and form new dense areas of sporulation less regularly and symmetrically, not necessarily of the *Alternaria* type though some of these may again arise.

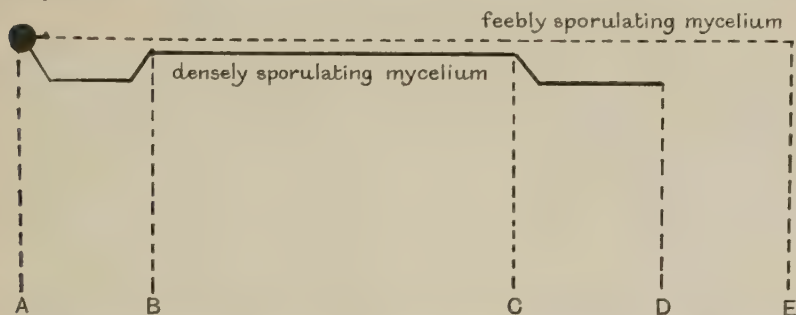
Unless, therefore, single spores are selected, there will be no definite central *Alternaria* area. By continuous selection of these spores, the

character can be handed on. In polysporous cultures it disappears from these plates, though, as has been shown experimentally, it may arise again when *B* spores are taken from the dense heads of a type *A* colony of polysporous origin.

DISCUSSION

The strain of this fungus as originally isolated has proved of peculiar interest from several points of view.

In the first place it reveals, when cultured on dilute media, that it regularly forms two kinds of spores, which give rise to two different types of colony, referred to as types *A* and *B*. The *B* type, however, is transitory and always reverts to the *A* type. The *A* type in turn repeats the cycle.



Text-fig. 4. A = inoculation point; AB = central zone of submerged spores; BC = middle zone of dark radiating bands; CD = outer zone of submerged spores; E = margin of culture. ----- = feebly sporulating mycelium ————— = densely sporulating mycelium.

A colony of the *B* type which constantly forms the *A* mycelium somewhat recalls the "ever saltating" strain of *Diaporthe perniciosa* described by Horne and Das Gupta(3). This strain DH_C , which originated from another strain DH_B , has a slow growth rate, and regularly saltates into DH_F (which appears to be identical with DH_B), when the cultures reach a certain age. In the same way, the *B* type of *Stemphylium* colony which originates from the *A* type has a slower growth rate and regularly saltates into the *A* type of colony. The description "ever saltating" would seem to be equally applicable to the fungus under investigation. The saltation is cyclic in nature, but in a mixed population, colonies of type *A* will always predominate.

The presence of submerged spores is very noticeable in the cultures. Their distribution seems to indicate that they represent the densely sporing mycelium, when it is not present on the surface of the medium as it is in the bands of dark sporulation. It is significant that even when on the surface it is always at a lower level than the more feebly sporulating mycelium. In the central zone and beyond the dark

bands, most of the spores of the densely sporulating mycelium are submerged. Radial growth of this mycelium ceases some little time before that of the feebly sporulating mycelium.

The two mycelia in a type *A* colony would appear to occupy the relative positions, shown in Text-fig. 4, which represents a sectional view along the radius of a culture.

A point of interest to the systematist is that the *Alternaria* type of sporulation is obtained occasionally. This may be a saltant form from the densely sporing mycelium. An alternative explanation is that this is a mixed race in which there are several different *A*'s and several different *B*'s, some of these latter being of the *Alternaria* form. The occurrence of the original *Alternaria* colonies in such small numbers would appear to favour the former view. Once obtained, the *Alternaria* character occurs in successive re-culturings of the strain.

SUMMARY

A *Stemphylium* is described in which growth on dilute media revealed that radiating bands of denser sporulation were produced in every generation whether of monohyphal tip, or single spore, origin. Microscopic examination showed that in these bands extremely dense heads of spores were developed. The rest of the plate was occupied by a more feebly sporulating mycelium, below which there was much submerged spore development. These colonies are distinguished as the *A* type of colony.

Spores carefully selected from these heads gave rise to slow growing colonies, which at first showed the densely sporulating character. The feebly sporulating mycelium was, however, initiated near the centre from whence it grew out in sectors, and surrounded the area of dense sporulation; submerged spore development was pronounced in the sectors. These are described as type *B* colonies.

On comparatively few occasions, in the densely sporing regions of small colonies on dilution plates, spore production in chains has occurred, characteristic of the form genus *Alternaria*. The behaviour of spores from these regions of dense sporulation was similar to those from the original dark heads of spores.

Subcultures of the strain derived from the *Alternaria* type of colony by reversion have for seven generations produced colonies in which sporulation in chains, associated with abnormal development of submerged hyphae occurs. The *Alternaria* spores from these continue to give rise to *Stemphylium* in every generation.

Preliminary experiments on the growth rate and staling reactions of the two types of colony, indicate that the disappearance of the densely sporulating mycelium is due to slower growth rate associated with greater sensitiveness to staling products accumulating in the medium, as compared with the more feebly sporulating mycelium.

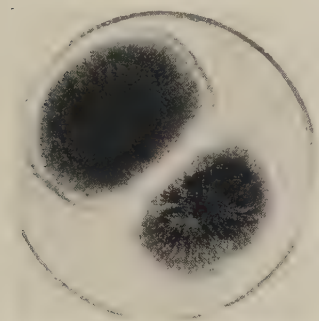


Fig. 1.

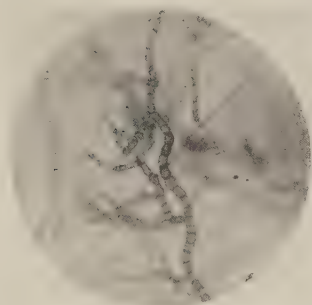


Fig. 4.

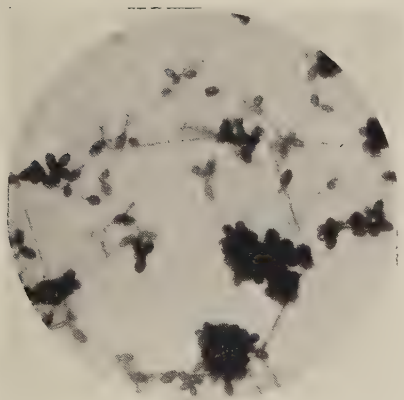


Fig. 2.

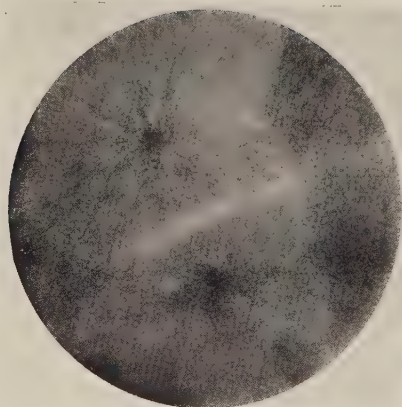


Fig. 5.

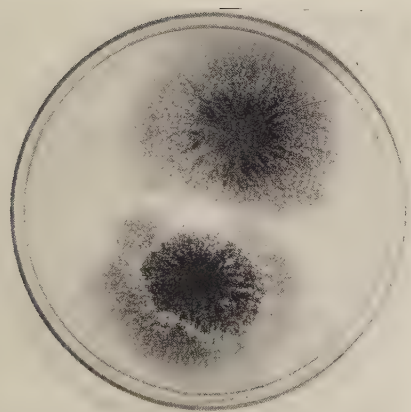


Fig. 3.

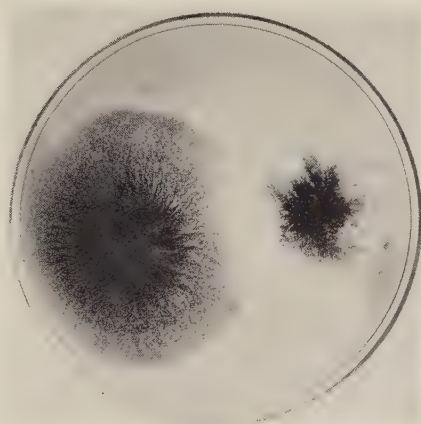


Fig. 6.

Stemphylium.

In both capacity for germination and rate of germination, the spores from the densely sporing mycelium are found to be inferior to those from the more feebly sporulating. Hence in a polysporous culture, which includes spores of both types, the *Alternaria* character does not show itself in the central area. Other densely sporing areas, which may or may not be *Alternaria*-like, arise from the more dominant mycelium derived from the paler spores.

My sincere thanks are due to Professor Brown and Mr Ramsbottom for their continuous interest and help in the working out of this problem.

EXPLANATION OF PLATE III

- Fig. 1. Two colonies of the *A* type on Brown's agar (diluted).
Fig. 2. Early stage in formation of dense heads of spores on 2 per cent. agar. Other sporulation much reduced.
Fig. 3. Colonies of *A* and *B* type contrasted. Brown's agar.
Fig. 4. Two colonies of *Alternaria*, with outgrowing sectors of *Stemphylium* on (modified) Dox's agar.
Fig. 5. Submerged swollen hyphae above which chains of spores arise in *Alternaria* strain.
Fig. 6. Two colonies (*Alternaria* strain) of the same age (twelve days) from dark spored and pale spored regions of *A*¹ type of colony.

LITERATURE CITED

- (1) BROWN, W. "Studies in the Genus *Fusarium*. IV. On the occurrence of Saltations." *Ann. Bot.* XL (1926), 223-43.
(2) WILTSHIRE, S. P. "A *Stemphylium* saltant of an *Alternaria*." *Ann. Bot.* XLIII (1929), 653-62.
(3) HORNE, R. S. and DAS GUPTA, S. N. "Studies in the genera *Cytosporina*, *Phomopsis*, and *Diaporthe*. I. On the occurrence of an "ever saltating" strain in *Diaporthe*." *Ann. Bot.* XLIII (1929), 420-35.

SOME EXPERIMENTAL STUDIES ON *PLEOSPORA HERBARUM* (PERS.) RABENH.

By M. ELLIS, *Assistant Lecturer in Botany,*
University College, Nottingham

(With Plate IV)

THE strain of *Pleospora herbarum* which forms the subject of the observations recorded here was isolated from plants of *Euphorbia Paralias* growing on sand-dunes near the mouth of the Dovey Estuary, Cardiganshire.

The fungus can be found upon living plants at any period of the year, and appears to be confined, in this area at least, to *E. Paralias*, since specimens of *E. portlandica* growing in close proximity are quite healthy.

The first indication of infection is the appearance of crimson spots, often reaching a diameter of 1 mm., upon the upper surface of the younger leaves. These spots are due to the development of anthocyanin in the epidermal and immediately subjacent cells. Lower down the stem, upon older, fading leaves, the spots are brown, whilst the dead leaves at the base of the plant bear minute black perithecia. The perithecial stage is preceded and accompanied by the conidial stage, and the stem as well as the leaves may be infected. Although a complete study of the life-history of the fungus was made, no previously unrecorded features were noted, and attention was chiefly focused on its behaviour in culture.

CULTIVATION ON ARTIFICIAL MEDIA

The fungus is easily obtained in culture upon malt extract agar (2 per cent. malt extract rendered solid by the addition of 2 per cent. agar).

Isolations were made and single-spore cultures obtained, and throughout the work subcultures from such single-spore isolations were used.

Cultures from single ascospores, kept at 22° C., show a whitish floccose mycelium within two or three days. After four or five days the mycelium begins to develop a dark pigment, and appears greenish brown when viewed from the underside of the dish.

The mycelium consists of much-branched septate hyphae, with fairly thick walls, and contains refractive oil globules and some glycogen. The older hyphae are dark in colour, owing to the development of a brown pigment in the cell walls.

The mycelium grows both on and below the surface of the agar. The aerial hyphae are profusely branched, giving the floccose appearance which is characteristic of the fungus in culture. The conidia are borne on the aerial hyphae, while perithecial primordia may be found either on or below the surface of the medium.

The conidia are of the *Macrosporium* type described by Miyabe(6) and seen on the surface of the leaves of *Euphorbia*.

Perithecial primordia, which begin to appear in cultures five to seven days old, are soon distinguished by the increased quantity of dark pigment in the cell walls; in cultures about ten days old they are visible to the naked eye as dark dots on the mycelium.

The earlier stages can easily be seen in preparations made by picking off small portions of the mycelium, staining with erythrosin and mounting in glycerine jelly. The primordia stain a deeper pink than the vegetative hyphae. They are very numerous, but of the total number of primordia laid down, not more than 30 per cent. ever reach such a size that they are visible to the naked eye as black dots on the mycelium. Even of these, only 15 to 20 per cent. ever reach full size and maturity, *i.e.* about 5 per cent. of the total number of primordia formed.

The time taken for the perithecia to mature varies considerably, but cultures about a month old usually show a number of perithecia with fully-developed asci.

The malt-agar medium has been utilised as a means of maintaining the stock cultures, since it is easily prepared, and is one upon which the fungus remains vigorous for long periods. A similar malt extract solution, without the agar, has also been used, to ascertain the effect of a liquid nutrient upon the fungal characters. In this medium the fungus forms mycelium both on and below the surface. The submerged mycelium consists of delicate, sparsely-branched hyphae. Conidia and perithecial primordia are formed only on the aerial mycelium, which forms a mat on the surface of the liquid. An additional solid medium containing a mush made from uninfected leaves of *Euphorbia Paralias* with 2 per cent. agar was also used. On this only a sparse, colourless mycelium was formed. Within a week conidia were produced in abundance, but no signs of perithecial primordia were seen for about fourteen days. Eventually a few scattered perithecia were produced, which after five or six weeks' growth formed asci and ascospores.

GROWTH ON SYNTHETIC MEDIA

A synthetic medium similar to that used by Robinson(7) in his studies on *Pyronema confluens* was made up as follows; NH_4NO_3 ($M/2000$), MgSO_4 ($M/500$), KH_2PO_4 ($M/500$), maltose ($M/500$) rendered solid by the addition of 2 per cent. agar. On this medium (described in the

following experiments as medium *A*1) the mycelium is less profuse than on the malt extract agar. It is not so dark in colour, though still distinctly brown, and the perithecial primordia are less numerous than on the richer medium. A culture ten days old showed an average of fourteen primordia per sq. cm.; a culture three weeks old, about thirty-five per sq. cm. A culture two months old, examined under a low-power microscope, showed an average of forty primordia per sq. cm., of which only about half were visible to the naked eye. In the central part of the culture about five primordia per sq. cm. developed into mature perithecia (*i.e.* about 12 per cent. of the total number of primordia formed), while in the outer parts the mature perithecia were more numerous—about ten per sq. cm., or 25 per cent. of the total number of primordia laid down.

Thus it is seen that though on this medium a comparatively small number of perithecial primordia are laid down, the proportion of these which reach maturity is greater than on the richer medium. To determine whether the nitrogen or the carbon content of the medium constituted the limiting factor for the growth of the fungus, media were made up using the formula of *A*1 as a basis, but varying the nitrogen and carbon content. Cultures on synthetic medium *A*2, a variant of *A*1 containing less nitrogen (NH_4NO_3 , *M*/4000), indicated that decreasing the nitrogen content of the medium has little or no effect on the growth of the fungus. The growth in these cultures is a little sparser than on medium *A*1, but a distinct brown colour is developed in the mycelium, and rings of perithecia are formed, as well as abundant conidia.

Growth on synthetic medium *A*3, in which the nitrogen content is doubled (NH_4NO_3 , *M*/1000), appears no different from that on medium *A*1.

On synthetic medium *A*4, in which the sugar content is decreased (maltose, *M*/1000), conidia and rings of perithecia are formed, but the vegetative mycelium is sparser and quite colourless. The dark pigment is formed only in the conidia and the perithecial primordia, and in a few adjacent cells.

That the fungus is able to grow and form reproductive structures on media extremely poor in both carbon and nitrogen is shown by the fact that cultures on *unwashed agar*, without any added nutrient, show a scanty, colourless mycelium with abundant conidia, and rings of primordia a few of which become fully developed perithecia. Cultures on *washed agar* (washed for six hours in running water, followed by twenty-four hours in distilled water), with no added nutrient, show a still sparser mycelium and fewer perithecia.

Four sets of cultures, M_1 , M_2 , M_3 and M_4 , were made as follows, there being twelve cultures in each set; M_1 , on washed agar: M_2 , on washed agar with NH_4NO_3 (*M*/2000) added: M_3 , on washed agar

with maltose ($M/500$) added: M_4 , on washed agar with both maltose and NH_4NO_3 in the above proportions.

All plates were inoculated with single ascospores, and within two days these had germinated, an appreciable amount of mycelium being formed in M_3 and M_4 . In six days a sparse mycelium was developed in M_1 and M_2 , and in M_1 curious distorted hyphae were seen, probably the result of starvation.

The mycelium of M_2 was rather more profuse, and showed no signs of starvation.

After a fortnight, all cultures showed perithecial primordia, these appearing as black dots on the mycelium.

The mycelium of M_3 and M_4 was definitely greenish brown, but no pigment had been developed in the vegetative hyphae of M_1 and M_2 .

After six weeks, all cultures showed fully developed perithecia with asci and ascospores, as well as abundant conidia.

The cultures of M_1 had a sparse, colourless mycelium with tufts of distorted hyphae. The perithecial primordia appeared scattered, numbering about ten per sq. cm. Those of M_2 showed no distorted hyphae, and the perithecial primordia were more numerous, about fifteen per sq. cm. The vegetative mycelium, however, was colourless.

In the cultures of M_3 , the mycelium was much more dense, and distinctly dark in colour. The perithecial primordia averaged twenty per sq. cm. near the periphery of the cultures but were less numerous in the central parts. They were developed in more or less definite zones.

In M_4 the mycelium was more profusely branched than in M_3 , darker in colour, and showed definite rings of perithecial primordia, numbering fifteen to twenty per sq. cm.

From these observations it may be concluded that the increase or decrease by small amounts of the nitrogen supply makes very little difference to the growth of the fungus, which is able to grow, though poorly, in a medium almost devoid of nitrogen. Unwashed agar contains in itself sufficient nitrogen to prevent any signs of starvation in the mycelium.

The supply of carbonaceous food material, on the other hand, affects the fungus more profoundly. Unless a certain proportion (between $M/1000$ and $M/500$) be present, the dark pigment is not formed in the vegetative mycelium, but only in the conidia and perithecia themselves.

These results indicate that in *Pleospora* a high Carbon-Nitrogen ratio in the medium is a necessary factor for the formation of pigment, the fungus in this respect resembling the *Fusarium* studied by Brown (3).

Having established the fact that the available carbon content of the medium is of very great importance to the growth of the fungus,

a further series of experiments was attempted, to find the source of carbon most suitable for growth. A series of media was made up, having the general formula of A_1 , but substituting different sources of carbon, as follows: *A*, maltose; *B*, glucose; *C*, sucrose; *D*, lactose; *E*, manitol. These media were all liquid, no agar being added.

Ascospores and conidia germinated immediately in these media, and formed considerable mycelium within four or five days. The mycelium in cultures *A*, *B* and *C* was more profuse and darker than in *D* and *E*. Within a week, perithecial primordia were produced in *A*, *B* and *C*. The relative amounts of mycelium and the number of primordia formed may be represented by the following table:

Medium	After four days	Seven days	Fourteen days
<i>A</i>	× × ×	× × × <i>pp</i>	× × × <i>ppp</i>
<i>B</i>	× × ×	× × × <i>pp</i>	× × × <i>ppp</i>
<i>C</i>	× × ×	× × × <i>pp</i>	× × × <i>ppp</i>
<i>D</i>	× ×	× × <i>p</i>	× × <i>pp</i>
<i>E</i>	×	× × <i>o</i>	× × <i>p</i>

(× × = relative amount of mycelium formed. *pp* = relative numbers of primordia developed.)

A similar set of cultures was made up on filter paper soaked in the liquids, and showed very similar results. These results, however, were rendered suspect by the fact that in control cultures on filter paper moistened with distilled water, a certain amount of growth took place. A sparse, colourless mycelium was formed, and later a few scattered perithecia, indicating that the fungus is able to a slight extent to utilise cellulose as a source of carbon.

A few cultures were made on oatmeal agar (0.5 per cent. oatmeal flour in water, with 2 per cent. agar). In these cultures a scanty colourless mycelium was formed, producing abundant conidia and eventually perithecia. Distorted hyphae similar to those found on washed-agar cultures were seen, indicating a starved condition. The perithecia were few, but were produced in more or less definite zones. Some cultures were stained with iodine after two weeks' growth, and showed that the fungus completely consumes the starch from the medium as it covers it.

These experiments show that the fungus is able to utilise various sources of carbon, the most suitable, however, being such sugars as maltose and glucose.

Some trouble was occasioned by bacterial contamination at one point in the investigations, and it was thought that by suitably adjusting the reaction of the medium this might be avoided. The malt medium was first tested, and gave a *pH* value of approximately 6.0, while the liquid media *A-E* all gave a *pH* value of 7.8.

Although the trouble caused by bacteria was eliminated without the necessity for alteration of the media, this suggested the idea of

finding the possible range of acidity and alkalinity within which growth of the fungus could take place.

Accordingly a series of media was made up from the formula of medium A_1 , but with pH value ranging from pH 2.5 to pH 9.2. The method adopted was to prepare the agar, sterilise it in test-tubes, investigate the pH by the potentiometer at $38^\circ C$. and apply the temperature correction. The media of pH 2.5, pH 3.0 and pH 3.5 were liquid, the others solid.

It was found that the fungus can grow to a very slight extent upon the pH 2.5 medium. No sign of growth was apparent in these cultures for two or three weeks, but eventually a small colony, about 4-5 mm. in diameter, was developed. This colony consisted of tangled colourless hyphae, composed of short broad cells. No reproductive structures were developed.

On pH 3.0 the colonies were larger (2-3 cm. in diameter) but were still colourless, and showed no sign of reproductive structures until the cultures were over six weeks old. A few perithecial primordia then appeared, but these never matured. No conidia were observed. The vegetative mycelium was normal in appearance.

The cultures on pH 3.5 showed more or less normal development, but the colonies did not reach a great size. In three weeks' time a few scattered perithecial primordia appeared, and cultures a month old showed scattered perithecia (five to ten per sq. cm.) with fully developed asci and ascospores.

On pH 4.5 there was some development of the dark pigment, but this was more noticeable on the cultures of pH 5.9-7.6. On media of higher pH value than 7.6 the dark colour of the medium made observation of the pigmentation difficult.

On pH 9.0 growth was not so vigorous, and fewer perithecia were formed. Fully developed perithecia were found, however, in cultures a month old.

On pH 9.2 no growth occurred.

The relative amounts of growth and numbers of perithecial primordia formed, are shown in the following table:

pH value of medium	One week	Two weeks	Three weeks
2.5	o o	x o	x o
3.0	x o	xx o	xx o
3.5	x o	xx o	xx $\bar{p}$
4.5	xx o	xx $\bar{p}$	xxx $\bar{p}\bar{p}$
5.4	xx $\bar{p}$	xxx $\bar{p}\bar{p}$	xxx $\bar{p}\bar{p}$
5.9-7.2	xx $\bar{p}\bar{p}$	xxx $\bar{p}\bar{p}\bar{p}$	xxx $\bar{p}\bar{p}\bar{p}\bar{p}$
7.6-8.5	xx $\bar{p}$	xxx $\bar{p}\bar{p}$	xxx $\bar{p}\bar{p}$
8.8	xx $\bar{p}$	xxx $\bar{p}\bar{p}$	xxx $\bar{p}\bar{p}$
9.0	x o	xx $\bar{p}$	xx $\bar{p}\bar{p}$
9.2	o o	o o	o o

(x x = relative amount of mycelium formed. $\bar{p}\bar{p}$ = relative numbers of primordia developed.)

Thus growth of the fungus and development of perithecia attain their maximum between pH 5.9 and pH 7.2, indicating that the fungus prefers a neutral or slightly acid medium, though it is capable of growth upon a definitely alkaline medium.

After three weeks' growth, the pH values of the media were determined by the capillator method. In testing the solid media a piece of the agar was cut out, melted, and mixed with an equal amount of the indicator.

The results are expressed in the following table:

Original pH value of medium (A)	After three weeks' growth (B)	After four weeks' growth (C)	Change in pH (C $\times$ A)
2.5	4.5	5.0	+ 2.5
3.0	5.0	6.0	+ 3.0
3.5	6.0	6.5	+ 3.0
4.5	6.5	7.0	+ 2.5
5.4	7.2	7.5	+ 2.1
6.2	7.5	7.8	+ 1.6
7.2	7.5	7.8	+ 0.6
7.6	7.8	8.0	+ 0.4
8.5	8.8	8.8	+ 0.3
8.8	8.8	9.0	+ 0.2
9.0	9.0	9.0	+ 0

It will be noted from the figures given in column 4, that where the initial pH of the medium is distinctly acid, the fungal metabolism is such that there is a shifting of the pH towards the point giving the optimum reaction for growth, this presumably being due to the formation of ammonia.

If, however, the initial reaction of the medium is alkaline, the formation of the alkaline by-product seems to be lessened, though the fungus does not appear to have the power of altering the reaction towards the optimum.

From the fact that the cultures on the pH 3.5 medium produced perithecial primordia only after three weeks' growth, by which time the reaction of the medium had been altered to pH 6.0, while those cultures on pH 4.5 required only two weeks before primordia could be formed, it appears probable that perithecia cannot be produced on a medium of a pH value below about 6.0.

The reaction of the sap from uninfected leaves of *Euphorbia Paralias* and from old leaves infected by the fungus was approximately pH 6.0.

ZONATION OF CULTURES

The development of perithecial primordia in concentric zones in cultures left on the laboratory bench has already been noted. The occurrence of zonation in fungal cultures has been described by many different investigators, and may be due to any one of many characters of the fungus, such as colour variations, branching of mycelium, and

density of sporulation, etc. These zonation effects have been attributed to various causes, such as the light relations, temperature variations, staling effects and mycelial crowding.

In *Monilia fructigena*, alternate zones of flat and conidia-bearing mycelium occur in culture. Hall⁽⁵⁾ attributes this zonation to variations in the light relation, since an exposure of three hours daily to light gives definite rings, while cultures kept in the dark at constant temperature showed no sign of zonation. Zones could also be produced by temperature variation, but were not comparable with those produced by day and night light variations.

Bisby⁽²⁾, working on *Fusarium discolor* also found that light is the chief conditioning factor causing zonation. Exposure for six minutes at 1 mm. from a 25 c.p. lamp suffices to cause zonation, but temperature is also a conditioning factor, since zonation may be produced by varying the temperature in absolute darkness.

In his work on strains of *Fusarium*, Brown⁽⁴⁾ noted some kind of zoning in colour effects, but the most striking zonation effect is due to the arrangement of the spore masses. The tendency to form zones appears to be a function of particular strains, since one strain forms a definite number of rings corresponding to the alternation of day and night, while another strain forms one zone of spores with no relation to the periodicity of light.

Stevens and Hall⁽⁸⁾ investigated the phenomenon of zonation for several fungi. In *Alternaria Brassicae* the end of each day's growth marks the edge of a zone which is intensified later by changes of colour. Here light is the chief conditioning factor. In *Ascochyta Chrysanthemi*, however, the zones are not due to light or temperature relations, but to the crowding of the mycelium, which shows alternate zones of profuse and sparse branching. The zonal formation of sclerotia in *Sclerotinia Libertiana* can also be attributed to crowding of the mycelium, rather than to temperature or light effects. The same conclusion probably applies to other fungi of a similar nature.

In *Pleospora herbarum* the zones are due to the formation of large numbers of perithecial primordia in definite concentric rings, separated by zones of vegetative mycelium relatively free from primordia. This gives the appearance of alternate zones of light and dark mycelium, owing to the production of a dark pigment in the primordia and associated hyphae. This pigment is also present to a lesser extent in the older vegetative hyphae. In the centre of each culture there is frequently a uniformly coloured area, with scattered perithecia, corresponding to three or four days' growth, and surrounding this area the alternate light and dark zones described. (See Pl. IV, fig. 1, of a culture on malt agar, fourteen days old, which has been left on the laboratory bench.)

The zonation effect is most pronounced in cultures growing on a

medium rich in carbohydrate, such as malt agar. On the various synthetic media the mycelium is less darkly coloured, though still distinctly brown in mature cultures, and the perithecia are produced in definite rings. Decrease of the sugar content in these media causes a decrease in the pigmentation of the mycelium, and so makes the zones less distinct. Cultures on unwashed agar without added nutrient show no trace of the pigment except in the conidia and perithecial primordia. Examination with a lens, however, shows that the primordia occur in definite rings. On washed agar with no added nutrient, the primordia are so few that it is impossible to trace any distinct zonation.

The fact that the zonation effect is seen only in cultures that are left exposed on the laboratory bench, led to the supposition that it is probably due to variations either of light or of temperature, rather than to staling effects in the medium, and it was decided to determine by experiment whether the light or the temperature relation was the conditioning factor. Accordingly a number of cultures were made on 2 per cent. malt agar, and treated as follows:

Set *A* was kept in the dark, incubated at 22° C. Here the temperature was constant, and the only variations of illumination were due to the temporary opening of the incubator for examination of the cultures.

Set *B* was incubated at 22° C., the dishes being wrapped in black paper so that both light and temperature factors were constant.

Set *C* was kept on the laboratory bench, the dishes wrapped in black paper, so that variations of temperature could affect the growth of the fungus, while the light factor remained constant.

Set *D* was also kept on the laboratory bench, the dishes wrapped in white, translucent paper. (This white paper was used in order to keep these cultures in the same conditions as the others with regard to humidity, air supply, etc.)

It was found that in cultures from sets *A* and *B* there was no sign of zonation (see Pl. IV, fig. 2). In both the mycelium appeared uniform in colour, with the perithecia more or less evenly scattered. The cultures of sets *C* and *D* both showed definite zonation.

Cultures kept at constant temperature, but exposed for a definite period (six hours) each day to daylight, showed no sign of zonation, nor did cultures kept at constant temperature but exposed to diurnal light variations.

From these results it may be concluded that variation of temperature is an important factor, if not the chief factor, in determining the zonation of cultures of this fungus, variations of illumination having no apparent effect. If this assumption is correct, it should be possible to induce artificial zonation by varying the temperature conditions.

Plates of 2 per cent. malt agar were inoculated and kept for alter-

nate two-day periods at 22° C. and 14° C. The diameter of each culture was measured at each change. The rate of growth of the mycelium was more rapid at 22° C. These cultures, examined after three weeks' growth, showed alternate light and dark zones of mycelium, the dark zones corresponding to the periods at the lower temperature. The dark zones consisted of mycelium with a greater development of the dark pigment and with more numerous perithecial primordia. A greater amount of aerial mycelium is also formed in these zones, giving the appearance of floccose rings on the upper surface of the culture, corresponding to the dark rings on the under surface (Pl. IV, figs. 3 and 4).

The experiment was repeated, the cultures this time being kept for alternating two-day periods at 28° C. and 22° C. The rate of growth was considerably greater at the lower temperature, from which it may be surmised that there is an optimum temperature for vegetative growth at about 22° C., any variation from this temperature causing a slowing-down of growth. (A further experiment, involving a series of cultures grown at temperatures ranging from 14° C. to 35° C., and measured at regular intervals, confirms this conclusion.)

These cultures, examined after three weeks' growth, also showed the alternating zones of light and dark mycelium, but the darker zones here corresponded to the periods at 28° C.

Other cultures were kept at a constant temperature of 22° C. for different periods, e.g. seven days, fourteen days, and then subjected to variations of temperature. No zones were formed in the central parts of these cultures (*i.e.* in the parts developed at constant temperature), but zones appeared as soon as the temperature was varied.

It was found that in cultures kept at 22° C. exposure for one hour each day to temperatures of 14° C., 25° C., 28° C. or 35° C. gave a definite zonation, the effect being most pronounced on exposure to 35° C.

It may therefore be concluded that there is an optimum temperature for vegetative growth at about 22° C. Exposure to higher or lower temperatures slows down vegetative growth, and this check is accompanied by the production of numerous perithecial primordia. The high concentration of the dark pigment in the walls of these, and of adjacent hyphae, gives the effect of the dark zones.

It is probable that any check to the vegetative growth of the mycelium will be followed by the production of increased numbers of reproductive structures, though all these may not necessarily mature.

A similar conclusion has been reached with regard to other fungi. Robinson (7), for example, finds that in *Pyronema confluens*, reproductive organs are formed as the result of any check upon vegetative growth. In *Pleospora herbarum*, such a check is not necessary to cause the

formation of reproductive bodies, but has the effect of increasing the number produced.

This conclusion is supported by the fact that in many cultures, especially on media poor in nutrient material, a greater number of perithecial primordia are laid down near the edge of the dish. This is especially noticeable in cultures on agar without added nutrient, where often only a few scattered primordia are formed in the centre of the culture, and a far greater number occur in a zone about 5 mm. wide near the periphery. Here vegetative growth is checked when the hyphal tips touch the glass, and an increased formation of reproductive structures follows.

OCCURRENCE OF SALTATION IN CULTURES.

The appearance of saltants in a single-spore culture of a fungus is of common occurrence, described by many investigators.

The factors causing saltation are in most cases unknown, though Barnes (1) has produced, experimentally, variations in *Eurotium herbariorum* by the effect of heat on the spores, and Brown (4) suggests that saltations in *Fusarium* are most likely to occur on media which combine high concentration with minimal staling capacity.

Saltation was first noted in *Pleospora herbarum* when certain cultures showed a sectorial effect (Pl. IV, fig. 5). Some parts of such a culture are composed of normal mycelium, bearing conidia and perithecia, while other parts are composed of colourless, much-branched hyphae forming a white, floccose mycelium which shows no sign of forming conidia or perithecia even after some weeks' growth, and which produces only a negligible amount of the dark pigment in the vegetative hyphae.

A large number of subcultures were taken from the normal part of such a culture, and of these about one-third showed the same appearance, the remainder being normal. Subcultures taken from the non-sectorial parent of the first sectorial culture were nearly all normal, but a few varied in the same manner. All subcultures taken from the white sectors produced the same white, highly floccose mycelium, incapable of forming conidia or perithecia, and in repeated subcultures on different media showed no sign of reverting to the normal, nor of giving rise to further saltations.

An explanation is thus offered of the occasional tufts of white, floccose mycelium sometimes seen in otherwise normal cultures (Pl. IV, fig. 3). These tufts occur very frequently on the rich malt extract media, and less frequently on the synthetic media. No signs of saltation were observed on the media extremely poor in nutrient material, but the saltant when grown on these media retained its characters.

Subcultures were made from these tufts, and from the normal mycelium near them, and again the two strains were obtained. Thus the white, non-fertile strain may arise in culture on any medium containing a sufficiency of nutrient material for normal growth, and may appear either in small tufts, or in definite wedges, giving the culture a sectoried appearance.

CONCLUSIONS

Pleospora herbarum, a facultative parasite upon *Euphorbia Paralias*, can easily be obtained in culture upon artificial media, both conidia and ascospores germinating readily. The fungus can grow and form reproductive structures upon media extremely poor in nutrient material, but on such media the mycelium is sparse (in extreme cases showing signs of starvation), and the perithecia are few, although abundant conidia are produced. The variation of the nitrogen content of the medium does not affect the fungus as profoundly as the variation of the carbon content. It is the latter which controls the pigmentation of the fungal mycelium, and to a large extent the production of perithecial primordia. Unless a sufficiency of available carbon is present, no pigment is produced except in the reproductive structures, and these are few in number; while the richer the medium, the more numerous are the perithecial primordia, and the more pronounced the pigmentation of the mycelium. In very rich media, the primordia are so numerous that only a small proportion can develop, whereas in poorer media the actual number of primordia formed is smaller, but a greater proportion of these reach maturity.

The fungus seems able to utilise any source of carbon, if necessary, but the most favourable media for growth and reproduction are those rich in such sugars as maltose.

Vegetative growth can take place on strongly acid or alkaline media, but no reproductive structures are developed on the former until the fungus has been growing for some time. During growth the fungus produces some substance of an alkaline nature, probably ammonia, which causes a decrease in acidity, and reproductive structures are not formed until the medium has attained a suitable reaction (roughly pH 6.0 which corresponds to the pH value of the sap of the host plant).

The zonation effect seen in many fungal cultures is very pronounced in *Pleospora herbarum*, giving the appearance of alternating zones of light and dark mycelium. This effect is caused by natural or artificial temperature variations, any variation from the optimum temperature causing a check to vegetative growth, which is accompanied by the formation of increased numbers of reproductive structures. This in turn involves increased formation of the dark pigment, giving the effect of dark zones in the cultures.

The production of reproductive organs as the result of a check to vegetative growth has been described for other fungi, and may well be so in *Pleospora herbarum*. It must be noted, however, that such a check is not necessary for their formation, but only causes an increase in the numbers formed.

The fact that in many cultures, especially on media poor in nutrient substances, an increased number of perithecia are formed near the periphery of the dish, supports the above conclusion.

Saltation frequently occurs in cultures, resulting in the production of a white, non-fertile strain which appears either in small tufts or in wedge-like sectors. No other saltations were observed. The new strain is easily isolated, and retains its characters on any medium. The factors which cause this saltation are not known.

Grateful acknowledgments are due to the late Professor Wilfred Robinson, of Aberystwyth, at whose suggestion the investigation was begun, and under whose direction most of the work was done; also to Professor H. S. Holden, of Nottingham, under whose direction the later stages of the investigation were carried out in the Department of Biology, University College, Nottingham. The writer wishes also to thank Miss Hall, of the Department of Plant Pathology, Imperial College of Science and Technology, South Kensington, for permission to read the manuscript of her work on strains of *Monilia fructigena*.

EXPLANATION OF PLATE IV

- Fig. 1. Culture exposed to day and night temperature variations, showing zonation.
 Fig. 2. Culture kept at constant temperature, showing uniformly scattered perithecia.
 Fig. 3. Culture kept for alternate two-day periods at 22° C. and 14° C., showing artificial zonation (upper surface of culture).
 Fig. 4. Culture kept for alternate two-day periods at 22° C. and 14° C., showing artificial zonation (under surface of culture).
 Fig. 5. Saltation. Culture showing appearance of new strain in wedges, giving sector appearance to mycelium.

REFERENCES

- (1) BARNES, B. "Variations in *Eurotium herbariorum* (Wigg.) Link, induced by the Action of High Temperatures." *Ann. Bot.* XLII (1928), 783-812.
- (2) BISBY, G. R. "Zonation in cultures of *Fusarium discolor sulphureum*." *Mycologia*, XVII (1925), 89-97.
- (3) BROWN, W. "Studies in the genus *Fusarium*. II. Analysis of Factors which determine growth-forms of certain Strains." *Ann. Bot.* XXXIX (1925), 373-408.
- (4) — "Studies in the genus *Fusarium*. IV. On the Occurrence of Saltations." *Ann. Bot.* XL (1926), 223-243.
- (5) HALL, M. P. "Factors controlling Zonation in Certain Fungi, with special reference to *Monilia fructigena*." Not published.
- (6) MIYABE, K. "On the Life-history of *Macrosporium parasiticum* Thüm." *Ann. Bot.* III (1889), 1-26.
- (7) ROBINSON, W. "The Conditions of growth and development of *Pyronema confluens* Tul. (*P. omphalodes* (Bull.) Fuckel)." *Ann. Bot.* XL (1926), 245-272.
- (8) STEVENS, F. L. and HALL, J. G. "Variations of Fungi due to Environment." *Bot. Gaz.* XLVIII (1909), 1-30.

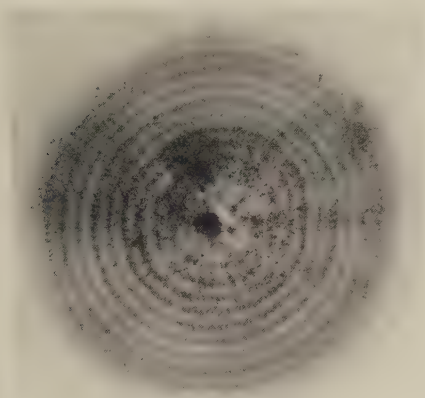


Fig. 1.

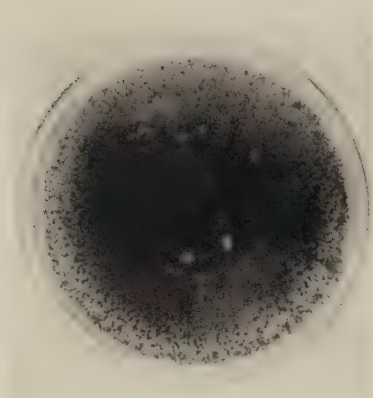


Fig. 2.

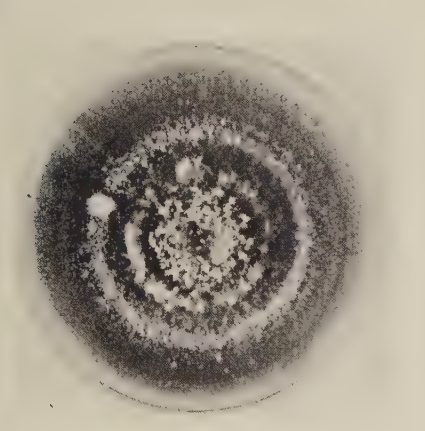


Fig. 3.

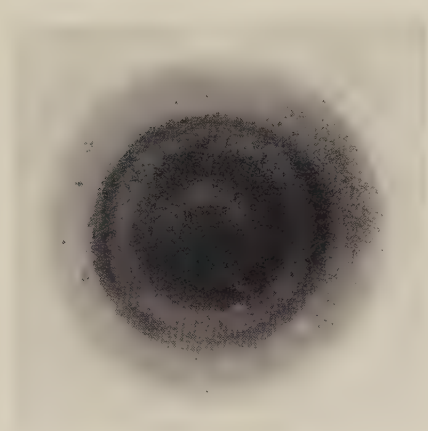


Fig. 4.

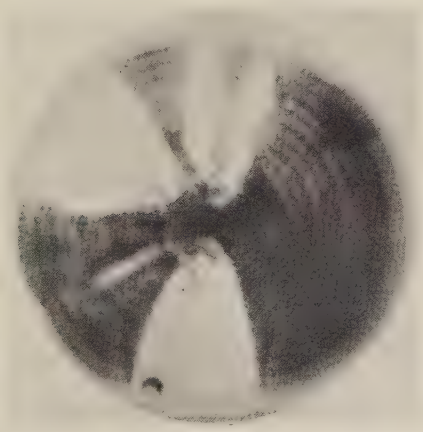


Fig. 5.

Pleospora herbarum (Pers.) Rabenh.

SALTATION IN THE GENUS *HELMINTHOSPORIUM*

By M. MITRA, PH.D., F.L.S.

(With Plate V and 3 Text-figures)

I. INTRODUCTION

THE variations described in a previous paper* on *Helminthosporium*, are of the nature of "modifications," *i.e.* are merely temporary alterations due to the environment and do not represent the full range of variability under cultural conditions, for the fungus sometimes "saltates" or "mutates" to a new form. Such genotypic changes have been described for the genus *Helminthosporium* and more particularly for the species *H. sativum* by various workers. Stevens⁽¹³⁾ showed that mutations were common in *H. sativum* and later Christensen^(4, 5) repeated and extended much of Stevens's work and corroborated his results.

All the cultures of *Helminthosporium* brought from India or isolated from Indian material have been carefully examined for the possible occurrence of saltant forms. A large number of these have been isolated, and their cultural features compared with their parent forms.

II. EXPERIMENTAL MATERIAL

The work was carried out with the same eight forms or species of *Helminthosporium*.

1. *H. sativum* Pamm., King & Bakke. (W.)†
2. *H. sativum* Pamm., King & Bakke. (B.)
3. *H. bicolor* Mitra. (W_p)
4. *H. halodes* Dresch. var. *Tritici* Mitra. (W_{cp})
5. *H. frumentacei* Mitra. (P.)
6. *H. maydis* Nisik. & Miy. (G.)
7. *H. Sacchari* Butl. (S.)
8. *H. Oryzae* Br. de Haan. (R.)

At the beginning of the work the purity of all the eight fungi used was guaranteed by Brown's hyphal tip method⁽¹⁾. Stock cultures were maintained in tubes on Brown's synthetic agar.

III. ORIGIN OF NEW STRAINS

New strains have arisen in two ways:

(1) In the well-known "Sector" form. Subcultures from such a sector will often give a pure saltant form. The sector may begin from

* Mitra, M. "A Comparative Study of Species and Strains of *Helminthosporium* occurring on certain Indian Cultivated Crops." *Trans. Brit. Mycol. Soc.* xv (1931), 254.

† For simplicity these forms will be referred to in the text by these symbols.

the centre, or from near the margin, or may grow out beyond the parent colony in a fan-shaped manner.

(2) From colonies which do not show typical sectoring. Inocula taken at random from different places of the same culture will often, especially on certain media, give rise to saltant strains.

(1) *Sector formation in culture*

Sectors have appeared from time to time in the cultures. They occur on almost all media and more particularly on prune-juice agar and Brown's starch agar. The saltant is sometimes a small white non-sporing area or conversely may be dark and heavily sporing as compared with the white poorly sporing parent. The saltant may occupy a greater area of the plate than does the parent. Pl. V, fig. 1 shows a complex example of sectoring, in which numerous sectors appear round the margin of the parent culture.

The isolation of the saltant forms is often simple, the sectors apparently being free from admixture with the parent. Occasionally the saltant proved to be thoroughly mixed with the parent strain and repeated trial was necessary before this was eliminated.

Shallowness of medium is an important factor in sector formation. This was first noted by Christensen^(4, 5) who states that *H. sativum* mutates only on the shallow side of a sloped plate. Experiments similar to those of Christensen have been carried out with the eight fungi investigated. The following test has been carried out three times, with similar results. Plates of uniform size were poured, some with 60 c.c., others with 33 c.c., and others again with 15 c.c. of Brown's starch agar. Some of the plates were allowed to set in a sloped position, so that there was a deep and a shallow side. Sectors appeared most abundantly in the plates with 15 c.c. of medium and on the shallow side of the obliquely poured plates. They were less frequent on the plates with 30 c.c. of medium and did not occur at all on the deepest plates. Pl. V, fig. 1 is an example of the highly sectorized type of colony which may arise on a shallow medium.

The majority of the sectors so produced proved on isolation to give rise to saltant strains. Some of them when tested simply reproduced the parent. Such "false" sectors, as they may be called, are particularly well seen in cultures of the strain W_p (Pl. V, fig. 2). The sector-like outgrowths occurring round the edge of the colony merely reproduced the parent when subcultures were taken from them. These false sectors appeared more frequently on shallow plates or on the shallow side of plates which had been allowed to solidify in a sloped position.

Sectors were formed freely by five of the eight forms investigated; the exceptions were strains W_{ep} , P and G .

(2) *Saltation in cultures which do not show the sectored appearance*

Saltants may also be obtained from colonies which show no sectored appearance; inocula taken at random from different places of the same colony will often, especially on certain media, give rise to saltant strains.

The occurrence of isolated patches of altered mycelium within the body of a colony on Richards's solution agar was described by Brown⁽²⁾ for a strain of *Fusarium fructigenum* Fr. There was nothing to indicate the presence of such patches, and it was only by sampling the different portions of the parent culture that the existence of the differentiated areas was demonstrated.

Mohendra⁽⁹⁾ obtained saltants in *Alternaria tenuis* Nees from patches of fluffy growth which appeared in a colony showing no sector formation.

In *Helminthosporium* Drechsler⁽⁷⁾ and others have noticed the formation of differentiated patches in old cultures of *H. sativum*. These patches consist of masses of compact white hyphae which grow through the layer of sporophores and at first glance suggest contamination. They are, however, saltant areas.

Brown⁽²⁾ found that Richards's solution agar was a suitable medium for giving rise to saltant strains. The eight forms of *Helminthosporium* under consideration were tested on this medium. Single hyphal tip inocula of each were placed on plates of Richards's solution agar (standard formula). The cultures were kept in double Petri dishes in order to avoid outside contamination. As they grew, they were sampled from time to time at various marked places, *e.g.* pieces of aerial mycelium, spores, or fluffy growth patches were transferred to plates of Brown's agar and the strains present were determined from their appearance on the latter medium. Pl. V, figs. 3 and 4 show sectored appearances where an inoculum gives more than one saltant; in fig. 3 there are three saltants and in fig. 4 there are two in addition to the parent.

Except occasionally, there was no obvious saltation in the form of sectors in the parent colony. Nevertheless, it was found that inocula taken from different parts of the parent consistently behaved differently from one another. Thus spores or mycelium from one place always gave a saltant, while those from another gave only the parent form. Saltants were obtained very frequently from the fluffy tufts of growth which appeared scattered over the colony (Pl. V, fig. 5), though they might also arise from parts which showed no differentiation from the general mass of the parent culture. The number of saltants obtained from areas near the margin was much less than from the central region.

As the parent culture becomes older, the number of spots from

which saltants can be picked up tend to increase. A culture of *H. sativum* (wheat strain) when tested after nine weeks gave saltants from six places out of ten. After twelve weeks, fifteen places out of twenty tested showed the presence of saltants. After twenty-eight weeks the number had risen to twenty out of twenty-two tested. Twelve tests with *H. Sacchari* gave three and ten saltants after three and nine weeks respectively. Similar results were obtained for the other forms. The strain from *Panicum frumentaceum* and one of the wheat strains (W_{cp}) gave no saltants.

The area occupied by the patches of tufted growth increases with the age of the culture (Pl. V, figs. 5 and 6).

These results are similar to those described by Brown⁽²⁾ for a strain of *Fusarium fructigenum* Fr. The only difference is that with *Helminthosporium* saltation in the parent is often made visible by areas of different growth.

(a) *Effect of richness of medium.* To determine whether the strength of the nutrient medium influences the tendency to saltation, a series of experiments was carried out on Richards's solution agar of three concentrations. These may be represented as N , $N/50$, $N/100$, the first being the medium as ordinarily employed. It was observed that growth was most vigorous on the medium of full strength and that the tufts of so-called secondary mycelium were present to a much less degree, if at all, on the more dilute media. The results of sampling tests, made in the manner described above, are assembled in Table I. The number of tests for each fungus on each medium varied from ten to forty. To demonstrate the effect clearly the figures in the Table, which give the number of saltant cultures arising, are reduced to a common basis of forty tests for each.

Table I*. *Effect of richness of medium (Richards's solution agar) on saltation*

Fungus	N	$N/50$	$N/100$
W	25	14	10
B	32	12	10
W_p	19	20	16
W_{cp}	0	0	0
P	0	0	0
S	32	14	2
R	33	10	4

* For explanation see text.

The above figures show that in general the number of saltants obtained is much reduced by dilution of the nutrient medium. It will be noticed that no saltants were obtained from the strains W_{cp} and P . The strain W_p behaved in a peculiar manner. It gave rise to a number of sectors, but these all proved to be of the type referred to above as "false sectors."

Results similar to those obtained on Richards's solution agar were obtained with oatmeal agar, Brown's starch agar and prune-juice agar. On all these media patches of secondary growth representing saltant areas are likewise formed.

(b) *Effect of temperature**. Plates of various media were set up at different temperatures ranging from 10° C. to 35° C. It was observed that patches of differentiated growth never appeared below 15° C. and that they increased in number with higher temperature to a maximum at about 30° C. At 35° C. their number was less. As these patches when tested invariably produced saltant strains, it is clear that the frequency of saltation is directly influenced by temperature.

In the preceding account the appearance of saltants as sectors and as scattered patches over the parent colony have been described separately. It is probable, however, that both represent the same phenomenon, the condition of growth obtaining in the culture determining the manner in which the saltant area will grow.

(3) *Saltation in test tube cultures*

Saltation may occur from time to time in the process of carrying on stock cultures in test tubes, and when this happens the fact may easily be overlooked. Transfers from stock cultures may give a mixture of parent and saltant or may give the latter only. In extreme cases the parent may be entirely absent in the subcultures. There is obviously a great risk of preserving the saltant and losing the parent.

During this work, hyphal tip stock cultures were kept side by side on two media, viz. Brown's agar and oatmeal agar. The stock cultures were more stable on the former medium. All the forms of *Helminthosporium* showed a marked tendency to the production of sterile races and this probably explains why a culture may become sterile if kept long in culture. The effect is not due to the power of spore formation being lost, but to the replacement of the parent by a non-sporing form.

Stable cultures are probably best maintained on sterilised straw media (oats, barley, wheat, etc.). Cultures on these media, even after six or eight months, have invariably been found to give the parent strain free from any admixture with saltants. One may recommend therefore that, if the aim is to keep stock cultures unchanged, rich media such

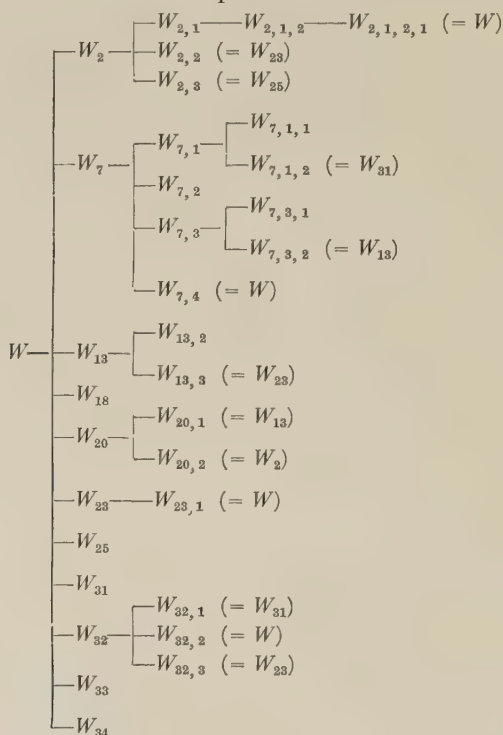
* Since this work was completed, Christensen(6) has published a paper in which he describes very similar results. As he apparently worked with comparatively shallow plates (18 c.c. of medium in 90 mm. diam. Petri dishes) the saltants appeared as distinct sectors. In my experiments, deep layers of medium were chiefly used and on that account the saltants took the form of the scattered patches. In a number of experiments in which shallow plates were used, the saltants appeared in the form of sectors and it was observed that the frequency of their occurrence was greater at 20–22° C. than at 15° C. or below. This is in complete agreement with Christensen's observations.

as Richards's solution agar, oatmeal agar, etc. should be avoided. Storage at a cool temperature, which also tends to reduce the tendency to saltation, is advisable for other reasons.

IV. NUMBER OF SALTANTS OBTAINED IN VARIOUS FORMS OF *HELMINTHOSPORIUM*

Not all the saltants obtained were new strains; the same saltant might recur time and again. The differences between saltant and parent might be slight from the beginning, and apparently vanish after some recultures. Such forms were discarded. Some saltants, though distinct, appeared to be very unstable, either saltating again or reverting to the parent. A large number, however, have proved to be sufficiently stable to allow of their culture and comparison over several generations.

Table II. *Illustrating origin of saltants from wheat strain of Helminthosporium sativum*



H. sativum, wheat form, has shown the most complex behaviour. Altogether sixty-three distinct saltants were isolated. Of these eighteen apparently reverted to the parent by the third generation,

though whether this was really reversion or merely the loss of the saltant from a mixture of saltant and parent is not clear. Twenty-seven were rejected as being too little different from forms already obtained to warrant their further culture. This left nineteen distinct forms. Table II illustrates the behaviour of this form. The system of indexing saltants is that used by Brown (2).

The table, p. 121, shows that several of the saltant strains may saltate further, sometimes giving rise to strains not distinguishable from others that appeared along another line. As all the forms were purified by the hyphal tip method, it is clear that reversion sometimes occurred. The most striking reversion was that of the strain $W_{2, 1, 2, 1}$ which arose from the parent form by four stages.

The total number of distinct strains (forty-eight altogether) obtained by saltation from the different parent forms of *Helminthosporium* is given in the following list:

1. W	19.	5. P	0.
2. B	10.	6. G	4.
3. W_p	4.	7. S	6.
4. W_{cp}	0.	8. R	5.

V. COMPARISON OF SALTANTS WITH THEIR RESPECTIVE PARENT FORMS

All the saltants were cultivated for a number of generations and the characters in which they differed from the parent were noted. The final comparisons were made on Brown's agar and Brown's starch agar.

The various saltants differed from their parent forms in the following respects:

(1) *Macroscopic cultural features*

The macroscopic characters vary greatly in the different saltants from one parent. As characters for taxonomic purposes, they are therefore of little value, and will be only briefly described.

The amount of *aerial mycelium* is extremely variable. Some forms have copious aerial growth, others have practically none, the growth consisting of creeping mycelium only; in some the aerial mycelium is dense, in others thin and cottony. The various saltants from the same parent may show every gradation in *colour* from white to quite dark. *Zonation* is well shown in some strains but not in others. The *linear rate of growth* is also very varied. Of all the strains in cultivation only one, a saltant from the barley form of *H. sativum* (B_3), produced *sclerotia*; none of the parents produced *sclerotia*.

(2) *Microscopic cultural features*

Intensity of sporulation. This, partly a macroscopic character, is very variable. Some saltants sporulate intensely, others from the same

parent are sterile. In a general way intensity of sporulation is indirectly proportional to the amount of aerial mycelium, which has been seen to be extremely variable.

Spore size. The majority of the saltants have a range of spore size similar to that of the parent form but a number of strains differed clearly from the parent in this respect. Thus of sixteen sporing strains derived from the wheat form of *H. sativum*, eleven showed a range of spore length similar to that of the parent and five did not. Again, while a saltant may resemble the parent in size of spore when tested on one medium, it may differ appreciably when grown on another. The amount of divergence shown by some of the saltants of *H. sativum* (wheat) on Brown's agar is indicated in Table III.

Table III. *H. sativum* (wheat). Range of lengths and widths of spore of certain saltants as compared with the parent strain

Strain	Range of spore length (μ)	Range of spore width (μ)
<i>W</i> (parent)	33.0-86.0	16.5-23.0
<i>W</i> _{7,2}	33.0-76.0	15.0-23.0
<i>W</i> _{7,3}	49.0-95.0	16.5-26.5
<i>W</i> ₁₃	23.0-89.0	19.8-26.5
<i>W</i> ₂₅	33.0-76.0	15.0-23.0
<i>W</i> ₃₄	56.0-106.0	19.8-29.7

Typical spores of four of the saltants of *H. sativum* are illustrated in Text-fig. 1.

The difference in spore length and width is more pronounced in some of the saltants of *H. Oryzae*. This variation is interesting taxonomically. Nisikado⁽¹²⁾ concludes, from a comparison of Japanese and American strains of this fungus, that they are morphologically distinct and that they may be different species. The chief differences concern the length and width of the spores. The range of variation in Nisikado's tables is no greater than that shown by the saltants of *H. Oryzae* in Table IV and it cannot indicate specific differences.

Table IV. *H. Oryzae*. Range of spore length and width of parent strain as compared with two of its saltants on Brown's starch agar

Strain	Range of spore length (μ)	Average length (μ)	Range of spore length (μ)	Average width (μ)
<i>R</i> (parent)	43.0-122.0	80.0	13.0-20.0	17.0
<i>R</i> ₁	63.0-172.0	123.0	16.5-23.0	21.0
<i>R</i> ₇	33.0-82.5	63.2	10.0-16.5	14.0

Text-fig. 4 shows typical spores of three saltants of *H. Oryzae*.

The variation in spore length and width found in *H. Sacchari* Butl. and some of its saltants possesses similar interest. The "eye spot" disease of cane is ascribed by various workers to *H. Sacchari* Butl., but Faris⁽⁸⁾ states that the form occurring in Cuba is different from that

described by Butler and Hafiz (3) from India, in possessing spores of average length 20μ greater, and in producing somewhat different pathological symptoms; he named it *H. ocellum*.

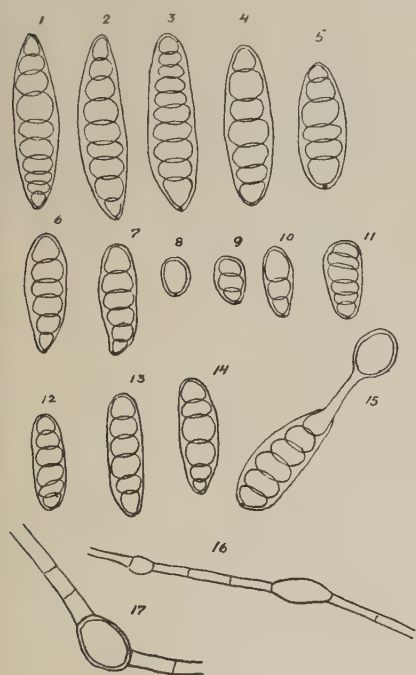


Fig. 1.

Text-fig. 1. Saltants of *H. sativum*. 1-3, strain B_3 ; 4-7, strain W_{25} ; 8-14, strain B_2 ; 15, strain B_5 showing formation of chlamydospore on germination of a spore; 16, 17, strain B_5 showing formation of chlamydospores in vegetative mycelium.

Text-fig. 2. Saltants of *H. Oryzae*. 1-9, strain R_1 . Spores 8 and 9 germinated at the distal end giving rise to a conidiophore-like structure. 10-17, strain R_7 ; 18-20, strain R_2 , 1.



Fig. 2.

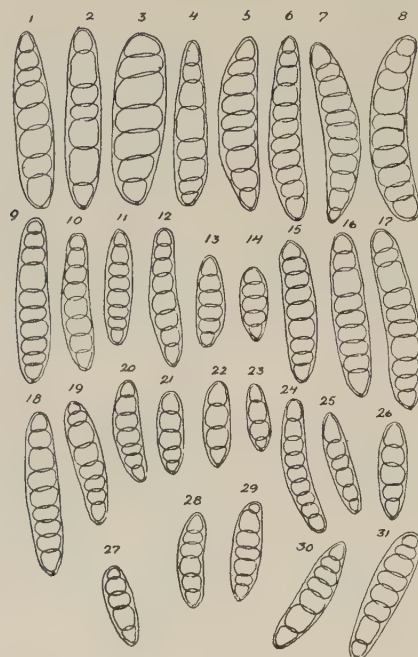
The variation among the saltants of *H. Sacchari* is illustrated in Table V.

Table V. *Variation in spore length and width of H. Sacchari Butl. and five of its saltants on Brown's agar*

Strain	Range of spore length (μ)	Range of spore width (μ)
S (parent)	33.0- 89.0	11.0-16.5
S_3	23.0- 56.0	10.0-15.0
S_5	23.0- 56.0	10.0-15.0
S_8	36.0-105.6	13.0-19.8
S_{10}	33.0- 56.0	10.0-19.8
S_{17}	33.0- 56.0	10.0-13.2

Text-fig. 3 shows typical spores of three saltants of *H. Sacchari*.

The figures given by various authors for the spore size of *Helminthosporium* on sugar-cane causing the "eye spot" disease are given in Table VI.



Text-fig. 3. Saltants of *H. Sacchari*. 1-9, strain S_8 ; 10-19, strain S_5 ; 20-31, strain S_{17} .

Table VI. *Spore measurements of Helminthosporium on sugar-cane as given by various workers*

Author	Range of spore length (μ)	Average length (μ)	Range of spore width (μ)	Average width (μ)
Breda de Haan (Java)	60.0- 80.0	—	9.0-12.0	—
Krüger (Java)	32.4- 90.0	78.5	9.0-14.0	12.5
Johnston and Stevenson (Porto Rico)	32.0- 90.0	—	9.0-14.0	—
Cook (Porto Rico)	45.0-110.0	—	12	—
Cook (Porto Rico)	22.0- 92.0	58.7	6.6-15.0	11.1
Drechsler (Cuba)	32.0-103.0	71.0	9.0-17.0	14.0
Faris (Cuba)	29.0- 94.0	68.9	9.0-21.0	12.7
Faris (Cuba)	32.0-110.0	69.0	6.8-20.0	12.7
Faris (Indian material)	18.8- 71.4	52.6	9.4-15.0	12.5
Butler & Hafiz (India)	35.0- 60.0	—	9.4-12.0	—
Mitra (India). Average for five media	30.0-105.0	68.3	11.5-18.5	15.3
Mitra (India). Strain S_8 average	36.0-112.0	81.0	13.0-19.8	16.0

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A study of the data given in Tables V and VI indicates that *H. Sacchari* Butl. and its saltants have a range of variation in spore dimensions sufficiently wide to include all the forms of *Helminthosporium* causing "eye spot" disease of sugar-cane.

It is clear therefore that length and width of the spores are not to be interpreted too narrowly as characters of taxonomic importance.

Degree of spore septation. This runs parallel with spore length and width, the larger spores being in general more highly septate, and conversely. The variation in degree of septation of *H. sativum* (wheat) and six of its saltants is illustrated in Table VII.

Table VII. *Variation in degree of septation of H. sativum (wheat) and six of its saltants on Brown's agar at 20° C.*

Strain	Range of spore septation
<i>W</i> (parent)	4-9
<i>W</i> _{7, 1, 1}	0-7
<i>W</i> _{7, 2}	3-8
<i>W</i> _{7, 3}	4-9
<i>W</i> ₁₃	2-7
<i>W</i> ₂₅	3-8
<i>W</i> ₃₄	2-8

The difference in the degree of septation is more pronounced in the saltants of *H. Oryzae* as shown in Table VIII.

Table VIII. *Septation of spores of H. Oryzae and some of its saltants on Brown's starch agar (100 spores of each counted)*

Strain	Range	Mean	Mode
<i>R</i> (parent)	3-9	6.2	6
<i>R</i> ₁	5-16	9.6	9
<i>R</i> ₇	2-8	4.3	5

Shape. The variation in length and width of the spores causes a slight but unimportant variability in shape. If the spores of the parent have rounded ends, so have those of the saltants. Other constant characters are, the degree of curvature (whether straight, slightly bent or crescentic), and the position of maximum width (whether midway or nearer one end).

Colour. Forms having spores of distinctive colours, transmit them to their saltants. In strains *W_p* and *G* the end cells are distinctly lighter than the intermediate ones, and this character appears in all the saltants.

Chlamydospores. These occur so rarely as to be of little taxonomic value. They have been observed in the strain *W*₃₁ of *H. sativum* (wheat) and in strain *B*₅ of *H. sativum* (barley) (Text-fig. 1).

(3) *Pathological behaviour*

One extensive test was carried out to determine the degree of pathogenicity of saltants of *H. sativum*. Eighteen saltants of the wheat form and ten of the barley form were tested. Each strain was inoculated into thirty seedlings of both hosts. The degree of attack was measured by the method of infection-rating used by McKinney⁽¹⁰⁾ and by Nisikado and Miyake⁽¹¹⁾. According to this method various degrees of infection are represented by standard numbers, a figure of 100 indicating strong infection of all the plants. Sample figures for a number of strains are given in Table IX.

Table IX. *Different degrees of virulence in saltants of H. sativum**

Strain	Tested on wheat	Tested on barley
<i>W</i> (parent)	83	50
<i>W</i> ₇	50	50
<i>W</i> _{7, 1, 1}	29	21
<i>W</i> _{7, 3, 1}	75	75
<i>W</i> ₃₄	19	19
<i>B</i> (parent)	83	75
<i>B</i> _{1, 1, 1}	15	19
<i>B</i> ₄	83	92
<i>B</i> ₉	13	26

* For explanation see text.

The data obtained indicate that there is a considerable range in pathogenicity among the saltants, some apparently being much weaker than the parent. Such a comparison is to be regarded as provisional. The establishment of different grades of virulence will require extensive experimentation, which it is proposed to carry out at a later date.

VI. SUMMARY

1. Of eight species or forms of *Helminthosporium* isolated from nature, all but two (one from wheat and the other from *Panicum*) produced saltants in artificial culture. Altogether forty-eight distinct saltants were isolated and studied.

2. New strains may arise as visible sectors. The tendency to sector formation is greater in shallow than in deep plates.

3. More often saltants arose as isolated patches scattered over the surface of the parent culture. Such areas of differentiation may or may not show a visible difference from areas which reproduce the parent type. They occur most frequently in old cultures, in cultures grown on rich media, and in cultures maintained at about the optimum temperature for growth.

4. A comparison between each parent and the saltants derived from it has led to the following conclusions:

(a) Macroscopic characters (including intensity of sporulation) are

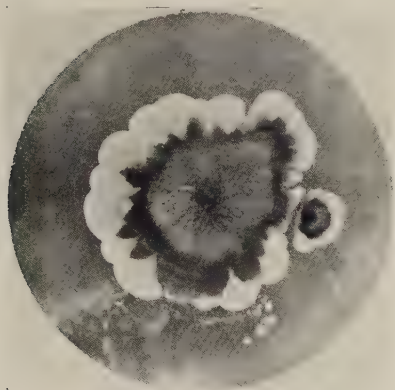


Fig. 1.

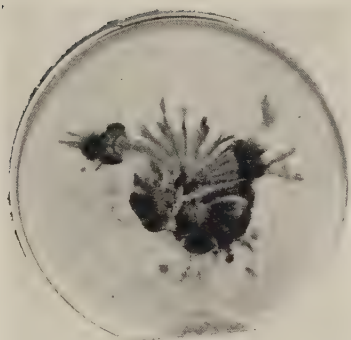


Fig. 2.

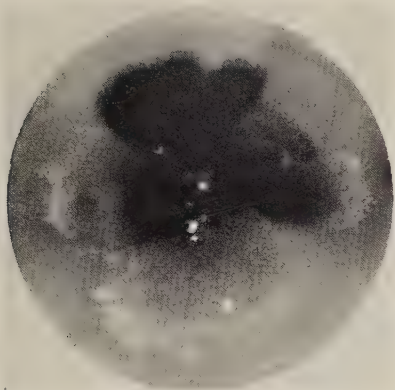


Fig. 3.

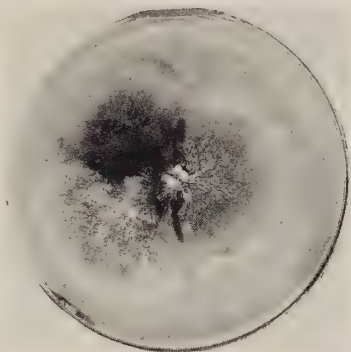


Fig. 4.

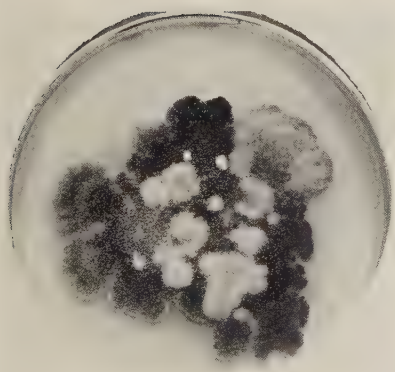


Fig. 5.

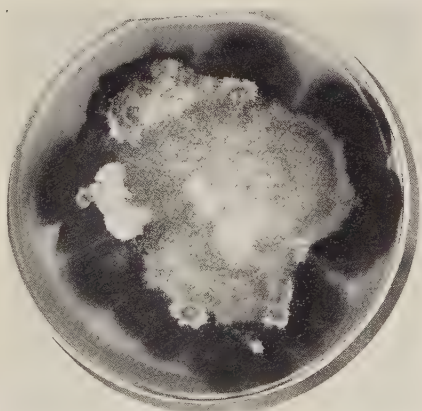


Fig. 6.

Helminthosporium.

extremely variable, so much so that scarcely any taxonomic value can be ascribed to them.

(b) There is much variation in the dimensions and septation of the spores though most of the saltants resemble the parent form in these respects. The shape and colour of the spores are fairly constant.

(c) Preliminary tests indicate that there is a considerable range of parasitic vigour shown by saltants of *H. sativum*.

The investigations were carried out under the direction of Prof. W. Brown, for whose advice and criticism I am under many obligations.

EXPLANATION OF PLATE V

- Fig. 1. *H. sativum* (barley). Large number of sectors formed on a shallow layer of Brown's starch agar.
 Fig. 2. *H. Sacchari*. Non-sporing type of sector. Three saltants appearing.
 Fig. 3. *H. Sacchari*. Similar to fig. 2, but two saltants appearing.
 Fig. 4. *H. Sacchari*. Appearance of several patches of scattered fluffy growth on Brown's starch agar. Cultures three weeks old.
 Fig. 5. *H. Sacchari*. Same as above, but cultures two months old and showing increase in the amount of secondary growth over the parent colony.
 Fig. 6. *H. sp.* (*W_p*) fan-shaped "false sectors" on a shallow layer of Brown's starch agar.

REFERENCES

- (1) BROWN, W. "Two Mycological Methods." *Ann. Bot.* xxxviii (1924), 401-4.
- (2) — "Studies in the Genus *Fusarium*. IV. On the Occurrence of Saltation." *Ann. Bot.* xl (1926), 223-44.
- (3) BUTLER, E. J. and HAFIZ, A. "Some new Sugar-cane Diseases." *Mem. Dept. Agric. India, Bot. Ser.* vi (1913), 181-208.
- (4) CHRISTENSEN, J. J. "Physiologic Specialization and Mutation in *Helminthosporium sativum*." *Phytopath.* xv (1925), 785-95.
- (5) — "Physiologic Specialisation and Parasitism of *Helminthosporium sativum*." *Univ. Minn. Agric. Expt. Station Bulletin* xxxvii (1926).
- (6) — "The Influence of Temperature on the Frequency of Mutation in *Helminthosporium sativum*." *Phytopath.* xix (1929), 155-62.
- (7) DRECHSLER, C. "Some Graminicolous Species of *Helminthosporium*." *Journ. Agric. Res.* xxiv (1923), 641-737.
- (8) FARIS, J. A. "Three *Helminthosporium* Diseases of Sugar-cane." *Phytopath.* xviii (1928), 753-74.
- (9) MOHENDRA, K. R. "A Study of the Changes undergone by certain Fungi in artificial Culture." *Ann. Bot.* xlii (1928), 863-89.
- (10) MCKINNEY, H. H. "Influence of Soil Temperature and Moisture on Infection of Wheat Seedlings by *Helminthosporium sativum*." *Journ. Agric. Res.* xxvi (1923), 195-218.
- (11) NISIKADO, Y. and MIYAKE, C. "Studies on two *Helminthosporium* Diseases of Maize, caused by *Helminthosporium turcicum* Passerini and *Ophiobolus heterostrophus* Drechsler (= *Helminthosporium maydis* Nisikado et Miyake)." *Ber. des Ohara Inst. für Landw. Forsch.* iii (1926), 221-66.
- (12) NISIKADO, Y. "Comparative Studies on *Helminthosporium* Diseases of Rice in the Pacific Regions." *Ber. des Ohara Inst. für Landw. Forsch.* iii (1927), 425-40.
- (13) STEVENS, F. L. "The *Helminthosporium* Foot rot of Wheat, with Observations on the Morphology of *Helminthosporium* and on the Occurrence of Saltation in the Genus. III." *Dept. of Reg. and Educ., Div. Nat. Hist. Surv.* xiv (1922), 77-185.

LICHENS FROM NORTHERN INDIA

BY A. LORRAIN SMITH

British Museum

THE lichens enumerated were sent to me by Professor Chaudhuri, Lahore University. Most of them were collected by his student, G. L. Chopra in various districts of Northern India. They have been listed according to the arrangement and nomenclature adopted by A. Zahlbruckner in his *Catalogus universalis* in which full references will be found. Unusual value is given to the collection by the careful notes as to locality and habitat. It has been impossible, unfortunately, to identify all specimens. In some the material was too scanty, in others there was imperfect or no fructification.

PYRENOCARPEAE

DERMATOCARPACEAE

- Dermatocarpon Moulinsii* A. Zahlbr. Col. A. C. Joshi, July 1929. On exposed stones, Kausernag, Kashmir, 12,000 ft. 34.—Col. H. Chaudhuri, June 1922. On exposed stones, Verinag, Kashmir, 7000 ft. 35 and 36.
D. miniatum var. *complicatum* Th. Fr. Col. Rev. E. Blatter, March 1927. On soil, Wasirastan, 2650 ft. Colour grey-black. ? no fructification. 50.
D. rufescens Th. Fr. Col. Rev. E. Blatter, March 1927. On calcareous soil, Wasirastan, 4250 ft. No fructification. 44.—Not determinable, probably the same as 44. On soil, Wasirastan, 2650 ft. 48.
Endocarpon pusillum Hedw. Col. G. L. Chopra, 1927. On walls, Gujranwala, Panjab. 119.

PYRENULACEAE

- Anthracotheicum variolosum* Müll.-Arg. Col. G. L. Chopra, July 1930. On bark, Darjeeling and its suburbs, 6500 ft. 75.

GYMNOCARPEAE

CYPHELIACEAE

- Tylophoron moderatum* Nyl. Col. G. L. Chopra, 1930. On bark, Sikkim, on the banks of the river Timi, 800 ft. 100.

GRAPHIDIACEAE

- Graphis scripta* Ach. Col. G. L. Chopra, July 1930. On bark, Darjeeling, 6500 ft. 83, 93.
Graphina Acharii Müll.-Arg. Col. G. L. Chopra, July 1929 and 1930. On bark, Darjeeling and suburbs, 6000–7000 ft. 43, 96.—Col. G. L. Chopra, July 1930. On bark, Martam, Gantok Road, Sikkim. 84.
Phaeographina exserta Müll.-Arg. Col. G. L. Chopra, July 1930. On bark, Darjeeling, 6500 ft. 99.

CHIODECTONACEAE

- Chiodecton Philippinum* Vain. (?) Col. G. L. Chopra, July 1930. On bark, Calcutta (New Lake). 63.—On bark, Calcutta. 94.
 Both specimens resembling *Chiodecton sanguineum*; they are faintly tinged rose-red at the edge.

PYRENOPSISACEAE

Thyrea pulvinata Massal. Col. Rev. E. Blatter, March 1927. On rocks, Wasirastan, 3000 ft. 46.

COLLEMACEAE

Collema sp. Col. Rev. E. Blatter, March 1927. On rock, Wasirastan, 2650 ft. Colour black. 51.

Leptogium trichophorum Müll.-Arg. Col. G. L. Chopra, July 1930. On rocks in moist situations, Tiger Hill, 7852 ft. 65.

L. Menziesii Mont. Col. H. Chaudhuri, June 1923. On bark near streams, Verinag, Kashmir, 7000 ft. 2.—Col. G. L. Chopra, July 1930. On rocks in moist situations, Tiger Hill, Darjeeling, 7852 ft. 66.—On moist stones, Maha Nadi, 4120 ft. 89.

— f. *fuliginosum* Müll.-Arg. Col. G. L. Chopra, July 1930. On moist stones, Maha Nadi, 4120 ft. 90.

L. palmatum Mont. Col. G. L. Chopra, July 1930. On moist stones, Maha Nadi, 4120 ft. 87.

L. Moluccanum Vain. (?) Col. G. L. Chopra, July 1930. No fructification, rather doubtful. On moist stones, Maha Nadi, 4120 ft. 88.

L. caesium Vain. Col. G. L. Chopra, July 1930. On bark, Darjeeling, 6500 ft. 92.

PANNARIACEAE

Coccocarpia pellita Müll.-Arg. Col. G. L. Chopra, July 1930. On rocks in moist situations and on trees, Maha Nadi, 4120 ft. 64.

STICTACEAE

Lobaria pulmonaria Hoffm. Col. G. L. Chopra, July 1929. On mortar, on stones in water, Darjeeling, 6000–7000 ft. 3.—On trunks of trees, Darjeeling (Tiger Hill), 7852 ft. 4.

Lobaria sp. Not determinable. *K* + yellow then rubescens, no spores. 9.

PELTIGERACEAE

Solorina crocea Ach. Col. G. L. Chopra, July 1928. On soil, Dalhousie, Chamba Road, 7000 ft. 8.

Nephroma sp. Col. H. Chaudhuri, July 1922. On bark, Verinag, Kashmir, 7000 ft. Too little material for accurate determination. The specimen resembles most nearly *N. expallidum* Nyl. but differs in several particulars: the hymenium is up to 100 μ high, the spores vary from 22–27 $\mu \times$ 7–8 μ .

Peltigera venosa Hoffm. Col. G. L. Chopra, July 1928. On ground among mosses, Dalhousie, Chamba Road, 7000 ft. 5.

LECIDEACEAE

Lecidea (Psora) decipiens Ach. Col. Rev. E. Blatter, March 1927. On soil, Wasirastan, 4250 ft. 45.

Bacidia (Weitenwebera) hunana A. Zahlbr. *Sym. Sinicae*, III (1930), 113, var. *rugosa* A. L. Sm.

Thallus minute rugulosus. Apothecia saepe conferta, usque ad 1.5 mm. lata; sporae nonnunquam longiores, ca. 27–40 μ .

On soil, Darjeeling, 6500 ft. Col. G. L. Chopra. 40.

B. luteola Mudd. Col. G. L. Chopra, July 1930. On bark, Darjeeling, 6500 ft. 97.

CLADONIACEAE

Baeomyces roseus Pers. Col. G. L. Chopra. On mossy ground in moist shady places, Darjeeling (Tiger Hill), 6500 ft. 39.

Cladonia pyxidata Hoffm. Col. A. C. Joshi, June 1929. On mossy ground in shady places, Kausernag, Kashmir, 12,000 ft. 38.

- C. furcata* var. *pinnata* Vain. Col. G. L. Chopra, July 1929. Moist conditions on ground, Darjeeling, 6500 ft. 37.—Col. G. L. Chopra, July 1930. Shady soil, Darjeeling (Tiger Hill), 7852 ft. 54 and 76.
- C. squamosa* var. *muricella* Vain. Col. G. L. Chopra, July 1930. On soil, Darjeeling (Tiger Hill), 7852 ft. 56.
- C. pityrea* Fr. Col. G. L. Chopra, July 1930. On soil, Sikkim, 4000 ft. 60.—On soil, Darjeeling (Tiger Hill). 61.
- Stereocaulon ramulosum* Ach. Col. G. L. Chopra. On exposed stones, Darjeeling and Sikkim, 400 and 7000 ft. 41.
- S. quisquillare* Hoffm. Col. G. L. Chopra, July 1929. On bark, Darjeeling, 6500 ft. 42.

GYROPHORACEAE

- Gyrophora cylindrica* Ach. Col. G. L. Chopra, July 1929. On exposed stones, Darjeeling (Tiger Hill), 7852 ft. 33.

PERTUSARIACEAE

- Pertusaria multipuncta* Nyl. Col. G. L. Chopra, July 1930. On bark, Darjeeling, 6500 ft. 67 and 98.

LECANORACEAE

- Lecanora subfusca* Ach. Col. G. L. Chopra, July 1930. On bark, Darjeeling, 6500 ft. 106.
- L. subfusca* var. *chlarona* Ach. Col. G. L. Chopra, July 1930. On bark of *Excrea aglauca*, Calcutta (salt marshes). 109.
- L. rugosa* Nyl. Col. G. L. Chopra, July 1930. On bark, Darjeeling, 6500 ft. 86.
- L. carnulenta* Nyl. Col. G. L. Chopra, July 1930. On stones, Darjeeling, 6500 ft. 104.
- L. coilocarpa* Nyl. Col. G. L. Chopra, July 1930. On bark, Darjeeling, 6500 ft. 105.
- L. (Placodium) chrysoleuca* Ach. Col. G. L. Chopra, July 1929. On rocks, Kashmir, Kausernag, 12,000 ft. 18.
- Lecanora* sp. Col. G. L. Chopra, July 1929. On bark, Darjeeling, 6000–7000 ft. 30.
- Haematomma puniceum* Vain. Col. G. L. Chopra, July 1929. On bark, Darjeeling, 6000 to 7000 ft. 31.—Col. G. L. Chopra, July 1930. On bark of *Araucaria*, Darjeeling, 6500 ft. 107.

PARMELIACEAE

- Parmelia Camtschadalis* Eschw. Col. G. L. Chopra, July 1929. On bark, Darjeeling, 6000–7000 ft. (also on rocks). 19.
- P. cetrarioides* Del. Col. G. L. Chopra, July 1930. On trunks of trees, Darjeeling (Tiger Hill), 7852 ft. 55.
- P. cristifera* Tayl. (?) no soredia. Col. G. L. Chopra, July 1929. On rocks near water, Darjeeling (Kurseong spring), 7000 ft. 14.
- P. latissima* Fee (?). Col. G. L. Chopra, Darjeeling, Kurseong spring, 7000 ft. 11.
—And f. *isidiosa* Müll.-Arg.
- var. *sorediata* Hue. Darjeeling, Kurseong spring, 7000 ft. 12.

The determination of these three specimens is doubtful as there are no fructifications; the colour is peculiar and also the habitat "on rocks." All the specimens give a very distinct reaction. Medulla KOH $\pm$ red, a characteristic of *P. latissima*.

- var. *marmariza* Nyl. Col. G. L. Chopra. On rocks, Darjeeling (Kurseong spring), 7000 ft. A very distorted form. 13.
- P. Nilgherriensis* Nyl. Col. G. L. Chopra. On rocks, Kurseong spring (or suburbs), Darjeeling, 7000 ft. 15, 17 and 79.—On rocks, Gangtok, Sikkim, 5500 ft. 73.
- P. tinctorum* Despr. Col. G. L. Chopra, July 1930. On trees, Sikkim, 5000 ft. 57.
- Parmelia* sp. Col. G. L. Chopra, July 1930. On trees, Gangtok, Sikkim, 5500 ft. 70.
- This specimen is deep red in colour somewhat resembling *P. acetabulum* or *P. tabacina* to which it is also very similar, but, whereas *P. tabacina* has a colourless medulla the plant from Gangtok has a brown "tabacine" medulla. The

spores measure ca. $25 \times 10 \mu$ and are too large for *P. acetabulum*. As in other specimens it is impossible to decide if the colour is normal. There is a red reaction with KOH, and the medulla yellow then red.

Parmelia sp. Col. G. L. Chopra, July 1930. On rocks, Sikkim, 3500 ft. 72.

Parmelia sp. Col. G. L. Chopra, July 1930. On trees, Timi, Sikkim, 4370 ft. 74.

The latter possibly new. No reactions with K or CaCl. Spores ca. $16-20 \times 10 \mu$.

P. conspersa Ach. Col. A. C. Joshi, July 1929. On rocks, Kashmir, Kausernag, 12,000 ft. 16. The determination of this specimen is also somewhat uncertain: the material is too scanty.

P. homogenes Nyl. var. *vestita* A. Zahlbr. *Symb. Sin.* III (1930), III, 183. Col. G. L. Chopra, July 1930. On rocks, Sikkim, 5000 ft. 58. The medulla is tinged pale yellow. In parts the thallus is densely isidiose. No fructification.

Anzia physioidea A. L. Sm.

Thallus cinereo-glaucescens, decumbens, lineari-laciniatus; laciniae elongatae, usque ad 5 cm. longae, ca. 2 mm. latae, sparse et divaricati-ramosae, ad apices crenatae, ad marginem leniter incurvae; subtus albus, nudus vel pulvinulis spongioso-panniosis, fusco-atris sparse tectus; rhizinae sparsae vel crebrae, ad 5 mm. longae, ca. 135μ latae ex hyphis valde tenuibus conglutinatis formatae, ca. 5.5μ crassae omnino dense spongioso-pannosae. Apothecia non visa. Ad cortices supra muscineas emortuas.

Darjeeling, Tiger Hill, 7852 ft. 53. Col. G. L. Chopra, July 1930.

This species more or less resembling *Parmelia physodes* in colour and form is distinguished by the long rhizinae completely clothed by the spongy hyphal network characteristic of the genus, the whole about 0.5 mm. thick. It creeps loosely over straggling dead mosses and hepatics and the long rootlets serve as very efficient organs of attachment and possibly as water-holders. In the absence of fructification, the species has been placed under the older genus *Anzia* rather than under *Pannoparmelia*, the number of spores in the ascus being the distinction between the two.

USNEACEAE

Ramalina farinacea Ach. Col. G. L. Chopra, July 1929. On bark, Darjeeling, 6000-7000 ft. 20.

Usnea florida Wigg. Col. G. L. Chopra, July 1929. On bark, Darjeeling (Tiger Hill), 7852 ft. 21.

U. comosa Vain. Col. A. C. Joshi. On trees, Kashmir. 23.

Usnea sp. Col. G. L. Chopra, July 1929. On trees, Darjeeling (Tiger Hill), 7852 ft. 22.

Siphula ceratites Fr. Col. G. L. Chopra, July 1930. On soil, Timi, Gangtok, 533 ft. to 5500 ft. 116. Primary thallus a green crust on the soil.

BUELLIACEAE

Rinodina sophodes Th. Fr. Col. G. L. Chopra, July 1930. On bark of *Excrea aglauca*, Salt marshes, Calcutta. 101.

Pyxine coccifera Nyl. Col. G. L. Chopra, July 1930. On *Ficus elastica*, Parabatipur, D. H. Rly. 108.

P. retirugella Nyl. var. *subtestacea* A. L. Sm.

Thallus laciniatus, levis, subtestaceus, plagas adpressas formans, KOH—vel leviter flavescens, laciniae ca. 1.5 mm. latae elongatae subradiantes, inter se arcte adherentes, in centro breviores et subimbricatae, ca. 200 altae; thallus subtus nigricans, ad ambitum albo-testaceus, rhizinis nigris, minutis, sparse obsitus; medulla alba vel subtestacea KOH—rubescens. Apothecia sat crebre dispersa, adpressa, lecidina, brunneo-nigra, usque ad 1.5 mm. lata, hymenium superne nigro-fuscum, KOH tarde purpurascens; sporae $21-27 \times 9-11 \mu$.

On exposed rocks, Darjeeling, 6500 ft. 68. Col. G. L. Chopra, July 1930.

Differs from the species in the non-rugulose thallus, and the testaceous colouring (which may, however, be accidental) in the red reaction of the subgonidial layer with KOH and in the somewhat larger spores.

P. retirugella Nyl. var. *macrotheca* A. L. Sm.

Thallus KOH —, medulla + rubescens. Apothecia usque ad 3 mm. lata, acetate frequenter rotundo-lobata; epithecium tarde K purpurascens; sporae ca. $22-24 \times 9 \mu$. The thallus of this variety is similar to that of the species; the apothecia are larger and lobate and the spores larger. In both varieties the red reaction of the medulla with KOH is immediate, the cortex is scarcely tinged yellow. The slight purple reaction of the epithecium appears only after many hours.

Sikkim, 4000 ft. Col. G. L. Chopra, July 1930. 69.

My first intention was to describe these two varieties as new species and investigation of more specimens may prove them to be distinct though in most characters they resembled *Pyxine retirugella*.

PHYSICIACEAE

Anaptychia leucomelaena Massal. Col. G. L. Chopra, July 1929. On mosses and mountains, Darjeeling, 6000–7000 ft. 24.

A. hypoleuca Vain. Darjeeling, 6000–7000 ft. 29, 32.—Col. G. L. Chopra, July 1930. 110, 111.—On rocks, Gangtok (Sikkim), 5500 ft. 112.

A. hypoleuca var. *sorediifera* Vain. Col. G. L. Chopra, July 1929. On rocks, Darjeeling, 6000–7000 ft. 25, 27.—Col. G. L. Chopra, July 1930. On rocks, Sikkim, 4000 ft. 71. The specimens have well-marked soredia. The two former are here and there tinged with reddish brown; the latter (71) is a very striking specimen, it is densely sorediate in the centre tinged with a rubescent colouring, and the outer rim of the grey laciniae yellowish-red to a depth of about 2 mm. It would be interesting to know the cause of the colouring. There is a slight indication of similar colouring in some herbarium specimens in the British Museum, but nothing so well marked.

A. podocarpa Trev. Col. G. L. Chopra, July 1930. On rocks, Gangtok, Sikkim, 5500 ft. 113.

A. Corallophora Vain. Col. G. L. Chopra, July 1930. On rocks, Sikkim, 4000 ft. 52.

A. speciosa Vain. Col. G. L. Chopra, July 1929. On rocks, Darjeeling, 6000–7000 ft. 26, 28.—Col. G. L. Chopra, July 1930. On rocks, Darjeeling, 6500 ft. 110.

Physcia hispida Frege. Col. G. L. Chopra, July 1930. On rocks, Gangtok, Sikkim, 5500 ft. 115.

P. hispida Frege (with *Anaptychia hypoleuca*). Col. G. L. Chopra, July 1930. On rocks, Timi Road, Gangtok. 114.

P. setosa Nyl. Col. G. L. Chopra, July 1929. On trees, Darjeeling (Kurseong spring), 7000 ft. 10.—Col. G. L. Chopra, July 1930. On trees, Tiger Hill, Darjeeling, 7852 ft. 77.—Col. G. L. Chopra, July 1930. On rocks or trees, Sikkim, 3000–5000 ft. 59, 78.

TILLETIA TRITICI ON AEGILOPS

By I. REICHERT, PH.D.

(P.Z.E. Agric. Exp. Sta., Tel Aviv, Palestine)

IN connection with our experimental studies on the comparative resistance of the different wheat groups to bunt (*Tilletia Tritici*)⁽¹¹⁾, a contamination experiment of *Aegilops* varieties, a genus with fourteen chromosomes, and much discussed by reason of its relation to *Triticum*, was undertaken.

We learn from phytopathological literature that Vavilov⁽¹⁴⁾ experimentally infected two out of the three species of *Aegilops* used by him, namely, *Aegilops cylindrica* and *A. ventricosa*—*A. ovata* remaining unaffected. It was therefore of interest to see how the *Aegilops* species used by Vavilov, and others not employed in his experiments, would react towards the *Tilletia Tritici* strain occurring in Palestine.

A special circumstance favoured the realisation of this project. Mr Eig, who has written a monograph on *Aegilops*⁽²⁾, provided me with twenty species, out of the twenty-two representing the entire genus, for the experiment.

The grains of *Aegilops* were de-hulled and then contaminated in the usual way by shaking them in a container with bunt spores taken from wild emmer plants. The seeds were sown, ten of each species, in pots filled with light sandy soil. After sowing, the pots were watered. The date of sowing was December 15th. The following species were used: *Aegilops bicornis*, *biuncialis*, *caudata*, *columnaris*, *comosa*, *crassa*, *cylindrica*, *Kotschyi*, *longissima*, *ligustica*, *ovata*, *sharonensis*, *speltoides*, *squarrosa*, *triaristata*, *triuncialis*, *umbellata*, *uniaristata*, *variabilis* and *ventricosa*.

The result of the test was that all the varieties except one remained unaffected, the exception being *Aegilops ventricosa*, which showed 50 per cent. infection.

We see that of the nineteen species of *Aegilops* used, even *A. cylindrica*, infected in Vavilov's experiments, remained uninfected by the Palestine strain of bunt. *Aegilops ventricosa* became infected in both experiments. The discrepancy in the results of the tests may be explained by a difference in the virulence of the bunt strains used.

SMUT ON AEGILOPS

Tilletia Tritici has never yet been found in nature on *Aegilops*. Only two species of *Ustilago* were found on it, namely *Ustilago Passerini* Fisch. v. Waldh.⁽³⁾, which from his diagnosis, seems to be identical with *U. Tritici*, and *U. Schumanniana* P. Henn.⁽⁵⁾.

TRITICUM AND AEGILOPS

The infection of *Aegilops* by *Tilletia Tritici* may possibly be considered as confirming in some respects the view held by certain systematists and geneticists (4, 10, 12), that *Triticum* is nearly related to *Aegilops* and that *Triticum vulgare* originates from a cross between *Aegilops* and a durum wheat. The cytological analysis of hybrids of *Aegilops* and durum wheat, made in the U.S.A. seems to support this view (12); but I would prefer not to draw any such conclusion, as *Tilletia Tritici* also attacks rye and barley—genera not at all related to wheat.

TILLETIA TRITICI ON OTHER CEREALS

Koernicke in 1872, and Kühn in 1876, found rye naturally infected by a *Tilletia*, and described it as *T. secalis*. The same disease is very prevalent in Russia, and has been found also in western Europe. Koernicke also found a *Tilletia* on barley, and described it as *T. Hordei* (8). Schellenberg found that these two species are morphologically identical with *T. Tritici* and should therefore be cancelled (13). Gaines and Stevenson were the first to succeed in proving experimentally the identity of these species (6). Jaczewski, however, apparently as yet unacquainted with the latest results, defends the validity of *T. secalis* (7), basing his view on the negative results of Bubak's attempts to infect rye with *T. Tritici*.

My experiments with *Tilletia Tritici* on *Aegilops* show the need for caution with this fungus and the inadvisability of drawing conclusions as to its identity from negative results.

It appears that Ducomet (1) also has recently succeeded in infecting wheat with *T. secalis* from rye. The capacity of *Tilletia Tritici* to infect *Secale* and *Aegilops* in addition to *Triticum* is thus experimentally proved.

CONCLUSIONS

1. Twenty out of the total of twenty-two *Aegilops* species were contaminated with *Tilletia Tritici*, only one, *Aegilops ventricosa*, becoming infected.

2. The transmission of *T. Tritici* to *Aegilops* supports Schellenberg's assumption that the same fungus is identical with *Tilletia secalis* on rye and *T. Hordei* on barley.

REFERENCES

- (1) DUCOMET, V. "La carie du seigle." *Rev. Path. vég. et d'Ent. Agric.* xiv (1927), 193-4.
- (2) EIG, A. "Monographisch-kritische Uebersicht der Gattung Aegylops." *Rep. Spec. Nov. Regni Veget.* Berlin (1929).
- (3) FISCHER VON WALDHEIM, A. "Les Ustilaginées et leurs plantes nourricières." *Ann. Sci. Nat. sér. VI*, iv (1876), 190-276.

- (4) HACKEL, E. "Gramineae." *Engler u. Prantl, Nat. Pflanzenfamilien*, 2 Teil, II Abt. (1887).
- (5) HENNINGS, P. "Fungi Aethiopico-arabici." *Bull. de l'Herb. Boiss.* I (1893), 97-122.
- (6) GAINES, E. F. and STEVENSON, F. J. "Occurrence of bunt in rye." *Phytopath.* XIII (1923), 210-15.
- (7) JACZEWSKI, A. DE. "Le développement menaçant du *Tilletia secalis* Kühn en Russie." *Report of the Internat. Conf. of Phytopath. and Ec. Ent.*, Holland (1923), pp. 267-72.
- (8) KOERNICKE, FR. "Mykologische Beiträge." *Hedwigia*, xvi (1877), 29-30.
- (9) KÜHN, J. "*Tilletia secalis*, eine Korn Brand Form des Roggens." *Bot. Zeit.* xxxiv (1876), 470.
- (10) PERCIVAL, J. *The wheat plant*. London (1921).
- (11) REICHERT, I. "Comparative bunt resistance of wheat in Palestine." *P.Z.E. Agric. Exp. Sta. Bull.* 9 (1928).
- (12) SAX, K. "The cytology of *Triticum* in relation to genetics." *Proc. Int. Congress of Plant Sciences*, I (1929), 347-50.
- (13) SCHELLENBERG, N. C. *Die Brandpilze der Schweiz*. Bern (1911).
- (14) VAVILOV, N. *Immunity of plants to infectious diseases*. Moscow (1918).

ALTERNARIA GOSSYPINA (THÜM.) COMB. NOV. CAUSING A LEAF SPOT AND BOLL ROT OF COTTON

By J. C. F. HOPKINS, *Plant Pathologist*
Department of Agriculture, Southern Rhodesia

(With Plate VI and 7 Text-figures)

THE commercial cottons grown throughout Southern Rhodesia are of the American upland type and within recent years have been almost entirely reduced to one variety, known as U₄, which has been selected for its jassid resistant qualities and has replaced strains of "Improved Bancroft" which formerly enjoyed much popularity. A characteristic of both these cottons during the first few weeks of growth is a more or less severe spotting of the cotyledons and early leaves which is frequently mistaken by farmers for angular leaf spot (*Bacterium Malvacearum* E. F. Sm.). The lesions are minute at first, but are rendered conspicuous by a relatively broad margin of reddish purple which surrounds a pale brown, killed area (Pl. VI, fig. 1). When it first becomes noticeable, the whole affected area has a diameter rarely exceeding 0.5 mm. and the central brown tissue is barely perceptible without the aid of a lens. In 1927, material collected for microscopic examination showed the lesions to be invaded by hyphae of a fungus, an *Alternaria* being subsequently obtained in culture. Towards the end of the 1928 season, a leaf spot of mature cotton which had been growing under adverse conditions was observed and an *Alternaria* was isolated from diseased tissue, whilst again in 1929, *Alternaria* was isolated from a cotton boll affected by an internal rot. Recent study of these cultures and material has proved *Alternaria* to be the causal agent in each case, and has established the identity of all three isolations.

SYMPTOMS

On the leaves a light brown area appears in the centre of the small purple spots and, as the lesion increases in size, the purple margin persists and may be half as wide as the killed area when this measures 1 mm. in diameter. The spots usually maintain a circular shape, are zonate and may be as large as 1 cm. in diameter, but rarely exceed this size; an average of twenty measurements equals 0.6 cm. As the fungus activity ceases, so the invaded tissues assume a vandyke brown colour, due in part to the production of conidiophores and conidia on the upper surface and the characteristic purple disappears

from the margin to be replaced by dark brown or black. On old leaves a small white spot may appear in the centre of the killed tissue, but this symptom is of rare occurrence and may be due to the presence of *Phyllosticta* sp. (? *gossypina*) which is sometimes found associated with *Alternaria*. Two or three spots may coalesce to form a dead area 2×1 cm. in size, the original lesions being clearly defined by the presence of zonations and the lighter colour of the intervening tissue. In severe attack, the earlier formed leaves may become somewhat chlorotic and under certain conditions (as was demonstrated when excessive quantities of potassic fertiliser were applied to the soil or during a period of prolonged heavy rain) may be shed (Pl. VI, fig. 3).

Examples of natural infection of bolls have been brought to my notice, but it would appear from field observations that the disease is of rare occurrence on fruits which eventually mature; the fungus, however, may be commonly found discolouring the exposed lint of ripe bolls after periods of rainy weather. Material which was examined in the laboratory showed small, almost black, depressed lesions about 1 cm. in diameter situated on the line of suture of the carpels, about 2 cm. from the tip of the boll, resembling closely lesions due to *Glomerella Gossypii* (Southw.) Edg. or *Bacterium Malvacearum* E. F. Sm. In the interior, a slight yellowish discoloration of the lint was observed and a dry rot had commenced immediately below the boll wall in two adjacent loculi bordering the seat of infection. The opened bolls were placed on one side and within forty-eight hours a copious sooty deposit of spores had appeared upon the exposed lint which, as will be seen, is characteristic of the boll rot phase of the disease.

According to Jones⁽¹⁾, who describes an almost identical affection due to *Alternaria macrospora* Zimmerm. on native cotton in Nigeria, fungus infection of bolls is principally confined to those a few days old and is concerned in their early shedding. Similar conditions appear to obtain in Rhodesia and a *Macrosporium** has been reported from completely rotted young bolls which, however, remained attached to the plant; this condition has also been produced experimentally as will be described later.

A leaf spot and boll rot, in which the lint is discoloured and "compacted" and covered with a black sooty deposit, have been recorded on three occasions by Eyles⁽²⁾, who referred the causal agent to *Macrosporium nigricantium* Atk., but material is unfortunately not available for comparison of the two diseases.

* Unpublished.

INFECTION EXPERIMENTS

Plants of U₄ cotton were grown in pots in the glasshouse and subsequently placed outside under cages of book muslin, which served the dual purpose of affording protection against insect attack and acting as incubation chambers when the fabric was kept constantly wet. Three methods of inoculation with suspensions of *Alternaria* spores in sterile water were attempted upon bolls four days old and about sixteen days old, the surface of the bolls having been previously washed with 0.01 per cent. corrosive sublimate solution, followed by sterile water.

- (1) Upon uninjured bolls.
- (2) Upon bolls the surface of which had been scratched by a needle.
- (3) By introducing the inoculum into the interior of the boll with a fine needle.

Control plants were used throughout.

Young bolls were picked after four days and no disease was observed in those which had received no injury. Two out of three bolls in class (2) showed a faint yellow discoloration of lint in the loculus immediately beneath the point of inoculation, and fungus mycelium was detected in both the lint and the tissues of the carpel. No spores were observed. The bolls which had been inoculated internally exhibited a reddish brown, soft rot of the seed and a soft, pulpy, yellow rot of the lint. Mycelium was present in abundance, but no spores were seen. After fourteen days, no disease had developed upon the remaining uninjured bolls, but those which had been scratched superficially had dry, shrivelled carpels, whilst the interior consisted of a black powdery mass of disintegrated tissue and fungus spores. In one boll the rot was confined to one infected loculus, the remainder having developed normally, thus giving the boll a distorted appearance. All infected fruits, even though completely disorganised internally, remained firmly attached to the plant and were not shed by the time that healthy bolls had ripened and split.

Sixteen days' old bolls were unaffected in classes (1) and (2) after twenty days from the date of inoculation, but where the inoculum had been introduced into the interior, a yellowish brown rot of the lint had begun after five days. In ten days the rot had invaded about two-thirds of the contents of the inoculated loculus, a small portion of the lint still retaining its white colour; the remainder was affected by a light brown, dry rot which had also reached the seed and produced a brownish discoloration of the immature cotyledons. Fungus mycelium was observed in both "stained" and unstained lint and also in the interior of the seed but did not appear to be present in the carpel except in the tissues adjacent to the inoculation wound. Within

forty-eight hours of the opening of the bolls, the characteristic sooty deposit of spores appeared in patches on both diseased and unstained lint. After twenty days, the carpels of all infected loculi had become dry and shrivelled, but the majority failed to open. In a few, however, the lips of the carpels curled away from the sutures revealing the typical dry rot of lint and sooty deposits of spores which appear to be symptomatic of the disease (Pl. VI, fig. 2). The interior of the seed was usually completely disintegrated but the seed coat mostly remained firm and hard. It was black and could be easily crushed between thumb and forefinger exposing a sooty mass of spores superficially resembling the smuts of cereals.

Similar infection was obtained in preliminary experiments in glass dishes with very young and half-grown bolls, and it was again found that uninjured fruits resisted the fungus but that surface wounding induced infection in the larger bolls after a prolonged period of time. It therefore appears that there is distinct physiological resistance to *Alternaria* in the boll walls, for even under extremely favourable conditions for fungus attack, provided that the epidermis is not ruptured, resistance is still maintained, whilst, even when the latter condition is not fulfilled, sufficient time must elapse for autolysis to set in before invasion of the carpel tissues is possible. This is in agreement with the behaviour of *Phytophthora* (3) on certain varieties of cotton having marked field resistance, which become susceptible to attack after being kept for some twelve days in glass vessels, and indicates the limited use of this method of testing pathogenicity with any but quickly developing diseases.

Typical lesions were produced upon young, growing leaves by spraying on a suspension of spores by means of an atomiser or by placing fragments of hyphae and spores on the upper surface. A few *Alternaria* spots developed upon the leaves of the control plants and were attributed to natural infection, the disease being difficult to control on any plants grown in the open. The fungi were isolated from naturally and artificially produced lesions and found to be identical in culture.

MICROSCOPIC

A considerable polymorphism was exhibited by the spores taken from different parts of the cotton plant (*i.e.* lint and interior of the seed), and those produced in culture, but, on the whole, their dimensions and the production of long chains suggests an affinity with *A. tenuis* Nees. The presence of a long-beaked form would appear to place the fungus outside the *A. tenuis* group and a comparison with *A. macrospora* Zimmerm. shows it to be distinct from that species, so that it is proposed to refer the Rhodesian organism to *Alternaria gossypina* (Thüm.) comb. nov., since Mason (4) has pointed out that

the fungus on authentic material of von Thümen's (*Mycotheca Univ.* No. 1469 in Herb. Kew) is an *Alternaria*.

On the plant

The hyphae on the leaf are dark brown, slightly constricted at the septa, with cells averaging $20 \times 3 \mu$, mainly in the leaf tissues, but sometimes growing on the surface. The conidiophores are at first



Fig. 1.

Text-fig. 1. Group of conidiophores and spores on upper surface of the leaf.



Fig. 2.

Text-fig. 2. Cluster of conidiophores showing geniculations.



Fig. 3.

Text-fig. 3. Spores on leaf surface.

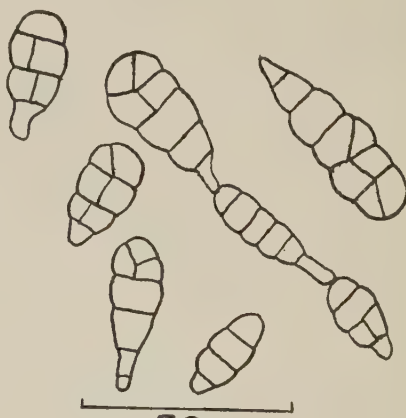
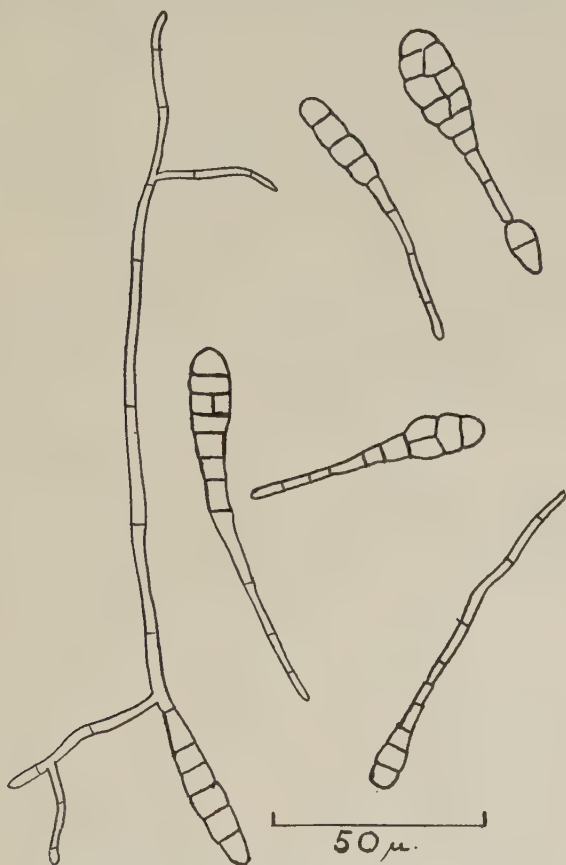


Fig. 4.

Text-fig. 4. Spores from interior of seed.

submerged and then erumpent, apparently bursting the epidermis, in clusters or single, $38-95 \times 6 \mu$, brown, septate, slightly constricted at the septa, geniculate and the basal cell swollen (Text-figs. 1 and 2).

The spores are dark brown, obclavate, with up to nine transverse and usually one or two longitudinal septa, constricted at the septa, $32-46 \times 12-15 \mu$ with a hyaline, sparingly septate beak $9-52 \mu$ in length, giving a total measurement of $41-98 \mu$ (Text-fig. 3). Catenu- late spores were observed in the seed and in cultures and are shown in Text-figs. 4, 5 and 6. Jones's (1) measurements for spores on the leaf



Text-fig. 5. Elongation of beak due to germination of terminal cell.
Spores from aerial mycelium of vigorous young culture.

surface are $34-50 \times 10-18 \mu$ with a beak $40-100 \mu$. It will be seen that the two spore measurements (with the beak), are in close agreement, but that the beak of the Rhodesian fungus does not attain the great length of the Nigerian one. On the lint, however, the measurements approximate to those of Jones's Trinidad fungus, the spore being $34-61 \times 12-15 \mu$ with a beak $12-73 \mu$, giving a total length of $46-134 \mu$, but the apparent long beak is made up of a certain

amount of hypha due to the germination of the terminal cell of the neck (Text-fig. 5). Spores obtained from the interior of seeds and cultures of different ages again possessed different dimensions and, in the table which follows, are compared with the Trinidad and Nigeria organisms.

Table I. *Comparison of spores from different parts of the cotton plant with those of Nigeria and Trinidad fungi*

Origin	Spore μ	Beak μ	Total length μ	Width μ
Leaf	32-46	9-52	41-98	12-15
Lint	34-61	12-73	46-134	12-15
Interior of seed	28-50	8-12	36-62	16
Young culture	35-54	29-46	64-100	10-15
Six weeks' old culture	25-52	23-65	45-117	12-16
Six weeks' old culture (perimeter)	23-41	9-16	32-57	14
Nigeria	34-50	40-100	74-150	10-18
Trinidad	37-60	80	140	13-18

In culture

After five days on potato dextrose agar at room temperature (20° - 27° C.) the growth measured 2.5 cm. in diameter, and was almost

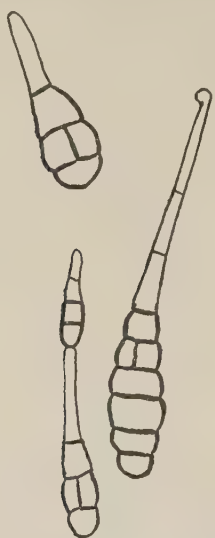


Fig. 6.

Text-fig. 6. Spores from centre of six weeks' old culture.



Fig. 7.

Text-fig. 7. Spores from edge of same culture.

completely covered by a thick, olive-green mass of spores with an area of white aerial hyphae, 1 cm. in diameter, in the centre. The hyphae were hyaline, vacuolate and granular at the edge of the colony,

becoming olive-green and then light brown. Marked constrictions of secondary hyphae were present at the points of branching. After six weeks, the culture was covered by an almost black sooty deposit of spores with an increased amount of white aerial mycelium in the centre of the plate. The hyphae were dark brown, constricted at the septa, the cells averaging $20 \times 3 \mu$, and considerable variation in spore measurements existed (Text-figs. 6 and 7) as indicated in Table I. After being transferred successively to corn meal agar, lima bean agar, oat juice agar and potato dextrose agar over a period of ten months, no pigment was produced in the medium, no diminution of spore production was noticed and no recognisable saltant appeared. The behaviour of the fungus in culture, therefore, differs from that of both the *Nigeria* (which ceases to form spores in culture) and the *Trinidad* (which is strongly chromogenic) organisms.

DISCUSSION

The characters of the leaf spots produced by *Alternaria macrospora*, a probably identical *Alternaria* in *Trinidad*, and Reichert's *Sporodesmium longipedicellatum* (now referred to *A. macrospora*) in *Tanganyika* are almost the same as those of the *Rhodesian* disease, except for a variation in the persistence of the purple margin which may well be determined by environment or differences in the varieties of the host plants, whilst the rot of young bolls is a symptom common to the *Nigerian* and *Rhodesian* diseases. The fact that shedding of "squares" is not caused by the latter can be attributed to the hereditary character of the U₄ strain of cotton, which has been selected for its low shedding qualities; the young bolls still remain attached to the plant even after boll-worm attack. As far as can be ascertained from available descriptions, the chief differences between the affections lie in the fungi concerned, two distinct species of *Alternaria* being involved.

Saccardo's description of *Macrosporium gossypinum* Thüm., from cotton stems, with broadly clavate, six to eight septate conidia, $36-40 \times 14-16 \mu$ borne on short, septate stalks, agrees well with the *Rhodesian* fungus in culture and on the leaf if it is conceded that the terminal beaks of the long-beaked form of spore were mistaken for proximal stalks and were probably omitted from the length measurement in the original description. That such an assumption is allowable is substantiated by the fact that the model form of the spores of the *Rhodesian* fungus is typically that of the *A. tenuis* group, particularly in the interior of the seed and in culture where long chains are formed in abundance. The presence of the long-beaked spore, which was consistently obtained in single spore cultures, would, however, place it outside this species and it appears desirable to apply the binomial *Alternaria gossypina* (Thüm.) comb. nov. at any rate until a closer relationship is established.

It was at one time thought that this fungus was a strain of *A. macrospora* owing to the similarity in the symptoms of the diseases produced and also to the presence of spores in the cotton lint whose length apparently attained as much as 134μ , thus comparing favourably with the Trinidad fungus. It has since been ascertained, however, that the very long extensions observed, both on the lint and in the aerial mycelium of some cultures, were the result of the germination of the terminal cell of the beak and that in reality the measurements included a certain length of germ tube. Such spores are shown in Text-fig. 5. In most instances the germ tubes are not as conspicuous as those in the illustration, but can usually be distinguished from the true beaks by the presence of a terminal swelling in the latter. Since Mason(4) has proposed to reserve the species *macrospora* for those *Alternaria* spp. on cotton whose spores (with beak) can attain a length of 200μ , it is certain that the Rhodesian fungus is distinct from the Nigerian and it is therefore of some interest to note the close similarity in the symptoms of the diseases caused by these two organisms.

My thanks are due to the Director and Staff of the Imperial Bureau of Mycology for considerable assistance.

SUMMARY

1. A common leaf spot of cotton in Rhodesia is attributed to *Alternaria gossypina* (Thüm.) comb. nov., which is also found associated with a rot of bolls.
2. Symptoms of the disease on leaves and bolls are described.
3. Details are given of inoculation experiments on both bolls and leaves by which the disease was produced on growing plants.
4. Microscopic characters of the fungus are compared with those of *Alternaria macrospora* causing a similar disease in Nigeria and Trinidad and certain cultural phenomena are noted.
5. Reasons are given for referring the Rhodesian fungus to *Alternaria gossypina* (Thüm.) comb. nov.

EXPLANATION OF PLATE VI

Fig. 1. Young *Alternaria* lesions on cotton leaf. $\times 1\frac{1}{2}$.

Fig. 2. Typical sooty deposit on lint in inoculated boll. $\times 1\frac{1}{2}$.

Fig. 3. Advanced stage of leaf spotting. The leaf is chlorotic and blotched with purple.
 $\times 1\frac{1}{2}$.

REFERENCES

- (1) JONES, G. H. "An *Alternaria* disease of the cotton plant." *Ann. Bot.* XLII (1928), 935-947.
- (2) EYLES, F. "Preliminary list of plant diseases occurring in Southern Rhodesia." *Bull.* 602, *Dept. Agric., S. Rhodesia* (1926).
- (3) HOPKINS, J. C. "Notes on the soft rot of cotton bolls in the West Indies caused by *Phytophthora*." *Ann. Bot.* XXXIX (1925), 267-280.
- (4) MASON, E. W. *Annotated account of fungi received at the Imperial Bureau of Mycology.* List II (Fascicle I). Imperial Bureau of Mycology, Kew (1928).

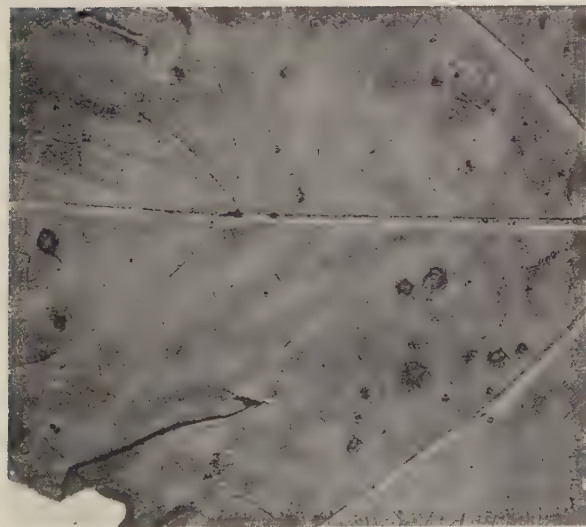


Fig. 1.

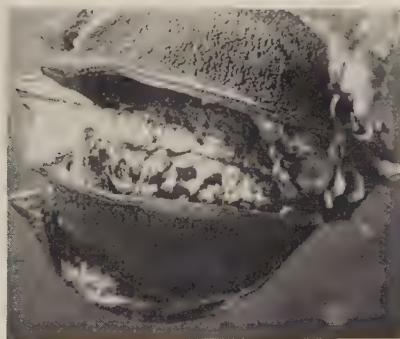


Fig. 2.

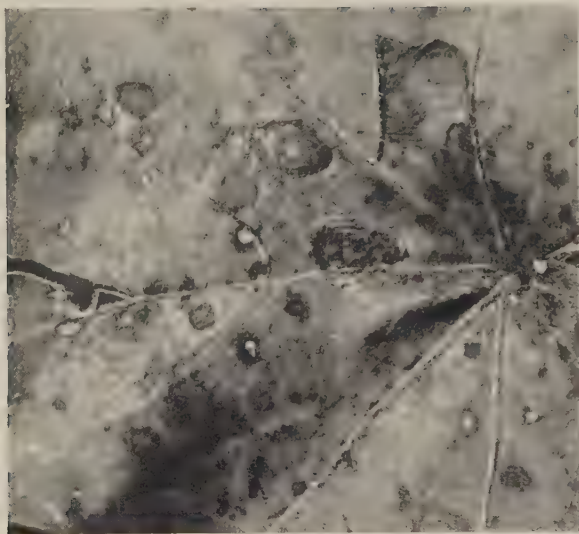


Fig. 3.

Alternaria Gossypina (Thüm.) Hopkins.

STANDARDIZATION OF COMMON NAMES OF PLANT DISEASES

By G. H. CUNNINGHAM, *Mycologist*

Plant Research Station, Palmerston North, New Zealand

IN the *Trans. Brit. Mycol. Soc.* xiv (1929), 140-77, appeared a paper by the Plant Pathology Sub-Committee of the British Mycological Society entitled "List of Common Names of British Plant Diseases." This is a valuable contribution since it is the first official attempt at the standardisation of common names for diseases of plants of parasitic or non-parasitic origin. The authors state in the introduction that the list was compiled with the object of selecting for each disease a single common name wherever possible, the use of which it was desired to encourage throughout the British Isles and the Empire. And further "It is realised that perfection has not been attained in the list now presented, which is to be regarded as to some extent provisional and tentative, and suggestions for the improvement of the list will be welcomed."

This latter request has led me to contribute this paper, especially as the list is intended for use throughout the Empire, of which New Zealand forms a part. Perusal of the list shows that there has been no system employed during its compilation in the choice of those common names accepted by the Sub-Committee. And as in fact no standard method exists, I am led to attempt the formulation of a few general recommendations, based on experience gained in attempts made during the past ten years to introduce a standard terminology into circulation among farmers, gardeners and fruit-growers in New Zealand (cf. *N.Z. Journ. Agric.* xxiii (1921), 163-66, 270-6; *Ibid.* xxiv (1922), 37-45, 96-102; *Fungous Diseases of Fruit-trees in New Zealand*, 1925), in the hope that subsequent lists may be arranged on some methodical basis.

OBJECT OF A COMMON NAME

Just as every common plant (and weed) has a common name, which conveys its identity to the farmer, fruitgrower or gardener as exactly as does its botanical equivalent to the botanist, so should it be possible to fix a common name to each disease with which the farmer, fruitgrower and mycologist has to contend. The object of a common name, then, is to offer the agriculturist and mycologist a label which must of necessity convey an exact and specific meaning, yet be readily pronounced by the layman. Among mycologists common names are frequently used as equivalents of the botanical names of those pathogens causing disease, or as indicative of the

symptoms produced by pathogens, by virus or by non-parasitic agency. It is evident that confusion must arise if an exact terminology is not in general use; and that such is the case is evidenced by reference to current literature, where it will be found that unless the pathogen is correlated with the common name (and listed under its botanical name), the reader is often left in doubt as to what disease is under discussion. And where a specific pathogen is unknown, the position is even more confused, as is exemplified by reference to the present potato virus position.

INCONSISTENCIES IN THE USE OF COMMON NAMES

There are no principles governing the use of common names, consequently confusion has been introduced into literature by this fact where (a) several common names have been applied to different symptoms produced by one pathogen on a single host. Examples occurring in the list are the names "Blossom Wilt," "Spur Blight," "Wither Tip," "Spur Canker" and "Brown Rot" for symptoms induced by *Sclerotinia cinerea*. Where (b) one disease has been attributed to several pathogens. Numerous examples occur in the list, among which may be cited "Leaf Spot," which is applied to similar diseases induced by twenty-two different pathogens, "Mildew" is applied to nine, "Downy Mildew" to seven, "Rust" to twenty-four, "Smut" to four, "Leaf Scorch" to five, "Root Rot" to six, etc. Or where (c) one pathogen occurring on several hosts (but producing similar symptoms on all) has acquired several names. Examples met with in the list are with the pathogen *Sclerotinia sclerotiorum*, which produces a disease which on potato is termed "Stalk Break," on tomato, artichoke, etc. "Sclerotinia Disease," and if on carrot becomes "Sclerotinia Rot." *Phoma Lingam* produces a disease which on turnip and swede is in the list labelled "Dry Rot and Canker," but if on cabbage and cauliflower becomes "Stem Rot." *Stereum purpureum* is found as "Silver Leaf" if on pome and stone fruits, but "Die Back" if on rhododendrons.

CHOICE OF A COMMON NAME

These examples show that under present conditions a common name has little significance, since it may be applied to a symptom, a group of symptoms, a pathogen, a group of pathogens or because of host distinctions.

If each common name were limited to the symptoms produced by any pathogen, or group of similar pathogens, then the choice would be narrowed to approximately a dozen names, as "Rust," "Smut," "Mildew," "Mould," "Blight," "Spot," "Rot," "Canker," "Scab," "Scorch" or "Wart." Such limitation would render any list unnecessary but would serve little other purpose, since these terms have

so general an application as to be useless other than as indicating groups of diseases. Alternatively, if every symptom were to bear a common name, irrespective as to whether one or several were produced by the same pathogen, each disease (using the term in its wider sense), would bear from one to half a dozen names, which would only obscure the issue, and make the practice of using common names quite unworkable. Therefore it is inadvisable to apply a common name to a symptom (an exception occurs where a disease is non-parasitic, or induced by an unknown pathogen).

If each pathogen were to bear a common name, irrespective of the number of symptoms it produced, or its host range, then the common name would come to have a significance equivalent to the botanical name of the pathogen, which would lend to it an exactness which at present is usually wanting. That such is the trend in modern literature is evidenced by the treatment of certain specific diseases in the list. Thus the two mildews of gooseberry, *Sphaerotheca mors-uvae* and *Microsphaera Grossulariae* are differentiated by the common names "American Mildew" and "European Mildew" respectively, and *Puccinia graminis* and *P. glumarum* are distinguished by the labels "Black Rust" and "Yellow Rust."

Such a practice would eliminate most of the inconsistencies in the list, save where the cause of a disease was unknown, or due to non-parasitic agency. Here a name would have to be chosen which was based on the characteristic symptoms of the disease, for there is no other method of differentiation.

In addition to this feature the choice of a common name has to be considered in relation to certain other matters. Thus if standardization is attempted, consideration must be given to names in use throughout the Empire, and those in most frequent use adopted, even though they are not in use in England, or New Zealand, as the case may be. For example, the disease produced by *Ophiobolus graminis* is given in the list as "Whiteheads," yet in Canada, South Africa, Australia and New Zealand it is known as "Take All." A second example occurs with *Venturia inaequalis* which in the list is placed under "Scab," but is known in Canada as "Apple Scab," and in Australia, New Zealand and (in part) South Africa as "Black Spot." In the first example it is evident that the name "Take All" should replace "Whiteheads" as it is more generally used, whereas in the second honours are about equally divided.

Appropriateness of a common name must be given due consideration. Examples of names which have lived many years because they were suitably chosen are "Silver Leaf," which describes the characteristic features of the symptoms induced by *Stereum purpureum*; "Coral Spot," which describes the colour of the fructifications of *Nectria cinnabarina*; "Loose Smut," which describes the features of

the sorus of *Ustilago Tritici*; and "Crown Rust," which indicates the feature of the teleutospores of *Puccinia coronata* (given as *P. Lolii* in the list). It will be noted that one of these names describes the symptoms, a second the colour of the fructifications as seen macroscopically, a third the characteristic features of the spore mass, while the fourth particularises the microscopic characters of the fungus. Examples in the list which I should consider unsuitable are "Spraing" for "Internal Brown Spot" or (one which is not given as a synonym in the list, but is in general use in New Zealand) "Brown Fleck" for a disease of potato characterised by the appearance of brown spots in the flesh of the tuber. "Blight" is likewise considered unsuitable for the disease of potatoes caused by *Phytophthora infestans*, on the grounds that the term has so general an application as to be specifically worthless; "Late Blight" is a preferable term. "Finger and Toe" is a poor substitute for "Club Root" for a disease produced by *Plasmodiophora Brassicae*, as it is not so descriptive of the condition, and scarcely as widely used. If "White Blister" is to replace the universally used "White Rust" for *Cystopus candidus*, as has been suggested by the compilers of the list, then "Downy Mildew" should likewise be abandoned for the reason that the pathogens listed under this name are not members of the Erysiphaceae.

Ease of pronunciation has a considerable effect upon the success of a common name, as is evidenced by the fact that the uncouth terms "Sclerotiose," "Ozoniose," "Hypochnose," "Podosphaerose," "Coniothyriose," etc. proposed by Stevens and Hall in their *Diseases of Economic Plants*, 1910, have not found favour save with a few workers in the United States. For this reason use of part of a botanical name of the pathogen is seldom successful, as unless the name is readily pronounced (as "Phoma Spot," "Botrytis Rot") it fails to find favour among farmers or fruitgrowers.

A standardized set of common names would prove acceptable to workers throughout the Empire only when a list was compiled in compliance with the expressed views of the majority. To bring this about I would suggest that the Chairman of the Sub-Committee approach the official Chief Mycologist in each Dominion, Crown Colony and Protectorate with a request that he (a) provide a list of those diseases of economic plants in his region; (b) indicate what in his opinion is the most acceptable common name for each disease; (c) give a synonymic list of common names for each, which are in use locally. From these particulars the Sub-Committee could then compile a list more in keeping with Empire requirements, and one in which moreover names could be employed which were based on the opinion of the majority of workers. Until such is undertaken, it is evident that the list under consideration cannot be regarded as having application outside Britain.

THE "SHAB" DISEASE OF LAVENDER

By C. R. METCALFE

(With Plate VII and 6 Text-figures)

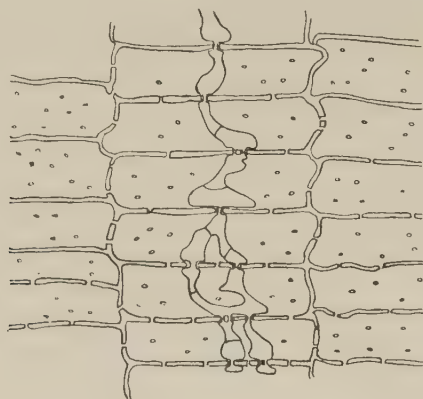
FROM about 1830 until immediately before the Great War certain parts of England were famous for the cultivation of high grade lavender for the manufacture of perfume. However, of recent years the industry has been crippled by the import of French lavender oil at a much lower price than that at which it can be produced in this country, and by a serious disease which has sometimes caused the death of all the bushes in plantations, several acres in extent. The first record of any trouble was in 1860 when many plants in the Hitchin district were killed following a severe frost, and from thenceforth it seems to have been present in most seasons to a greater or less extent. It has been attributed to over-manuring, to frost injury, and to the presence of too much essential oil in the plant; a waterlogged soil was also held responsible. The possibility that the disease might be due to fungal attack was first considered in 1892 when a brief description in Sawyer's *Odorographia* recorded that a "white fungus" developed on the roots of infected plants.

The disease, which now commonly passes under the name of "Shab," was first investigated scientifically by Brierley⁽¹⁾ who proved that it was due to the fungus *Phoma Lavandulae* Gabotto, and the present investigation has confirmed this, although other causes may sometimes give rise to symptoms that might readily be confused with those resulting from true "Shab" attack. Since then diseased lavender has been recorded in France by Chevalier in 1922, who, on very insufficient evidence, attributed the disease to *Pholiota praecox*, and in 1924 Lazare also recorded rotting of lavender in France in fields which had suffered from drought or in which there had been heavy applications of nitrogenous manure.

Following complaints about the disease received by the Ministry of Agriculture and Fisheries from a grower at Hitchin, I began a re-investigation of the disease in 1926, at the suggestion of Dr G. H. Pethybridge. Detailed observations were made at Hitchin on the disease as it occurs in the field, and other commercial lavender plantations in different parts of the country were visited. Thanks are due to Mr F. T. Brooks under whose supervision the work was carried out, as well as to the lavender growers who co-operated in the investigations.

EARLY SYMPTOMS OF DISEASE

The earliest symptoms of the disease can be seen between May and August when certain of the young shoots wilt and die, usually following a preliminary yellowing. The disease develops downwards from the newly infected shoots; lower branches become infected at their bases, and from these the disease spreads upwards into other branches which subsequently die in the same way; lastly, the roots are invaded. The general appearance of plants killed by the disease is shown in Pl. VII, fig. 2, the small dead plants in the foreground being in marked contrast to the background of healthy bushes.



Text-fig. 1. Thick hyphae in the medullary ray cells. $\times 300$.

It is common for one side of a plant to die before the disease is seen in the remainder, because the rootstock becomes infected before the fungus is able to invade the other side. When the healthy part of such a plant is infected it succumbs very rapidly.

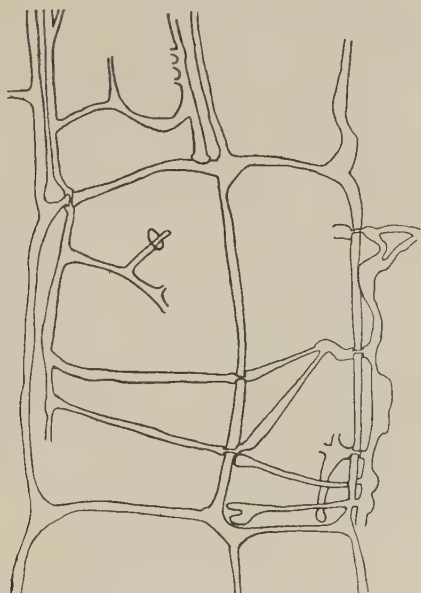
Densely crowded pycnidia are formed at a late stage of the disease chiefly on young twigs, and especially on those destined to bear, or which have borne, flower heads. The fungus is identical with the one Brierley called *Phoma Lavandulae* Gab. The average size of the spores is $4 \times 2 \mu$. Other *Phoma* spp. with larger spores have also been found on lavender but these have proved to be saprophytes.

Pycnidia of *Phoma Lavandulae* are sometimes produced on old lavender stems, more especially when the bark has been removed by some mechanical agency. They are also formed on the leaves but are rendered inconspicuous by the stellate hairs; they are clearly seen if the infected leaves are moistened with water or immersed in Eau-de-Javelle for a few moments. Although pycnidia are not commonly produced except on tissues that have actually been killed by the disease they have been met with from time to time on newly infected tissues.

The tissues of a diseased stem are brown or almost black, and where diseased and healthy tissues are continuous there is a distinct line of demarcation.

For microscopical examination freehand sections were stained with cotton blue in lacto-phenol, Delafield's haematoxylin, or gentian violet and congo red. All these stains were useful in differentiating the mycelium from the tissues. Usually cotton blue was the most satisfactory.

A branching septate mycelium ramifies in all the diseased tissues, but is most abundant in the vessels of the older stems where it passes



Text-fig. 2. Thin branched hyphae in the cells of the pith. $\times 300$.

from one vessel to the next only through pits in the walls. In the medullary ray cells, very thick, dark coloured hyphae are found (Text-fig. 1) which are connected with the mycelium in the vessels, and also with a mycelium of thin branched hyphae in the pith which also passes through pits in the walls (Text-fig. 2).

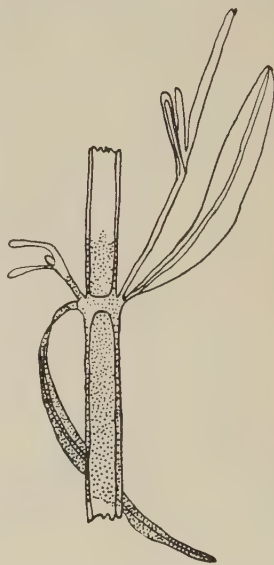
The brown appearance of the infected tissues is due to a discoloration of the cell walls. In transverse sections of young diseased stems, however, a considerable amount of gum is visible, especially in vessels in which the mycelium is first established. Sometimes a few isolated pith cells also become filled with gum.

MODE OF INFECTION

Extensive field observations were made to determine the mode of infection by the fungus. A large number of twigs which were suspected to be infected were observed at frequent intervals; the results show that there are two ways in which infection can occur. In the first the earliest symptoms of disease are seen when the tissues at or near a leaf axil on a young stem turn brown. Later the leaf turns bright yellow, usually only one leaf of a pair at a node becoming infected. From the infected leaf axil the yellowing extends up and down the stem frequently without passing to the other side. This leaf now becomes brown, curls round, and dies (Text-fig. 3). Although infections of this type are frequent in leaf axils, they may occur anywhere on the lamina of a leaf, or sometimes on an internode of a young stem. The yellowing extends rapidly and leaves at successive nodes wilt and die (Pl. VII, fig. 1).

The second method of infection is through wounds. These are made very extensively when the crop is harvested and also as a result of clipping. Here no disease symptoms can be seen until the young shoots immediately below the wound develop.

Once wilting has started the disease develops in the same way. Usually a period of from three weeks to a month or so elapsed between the first appearance of the disease and the death of the twig. About two weeks later abundant pycnidia of *Phoma Lavandulae* were formed. The speed with which the disease developed from this stage onwards varied in different plants. Some were killed within a few months, but in others the disease appeared to become retarded, and occasionally plants that were known to be diseased, during the winter, exhibited no symptoms that could be recognised with the naked eye. In the following season, however, they succumbed to the disease very rapidly. No evidence that the fungus becomes held up by a gum barrier or similar obstruction has been found, and it is thought that the phenomenon is due merely to the slow growth of the fungus during winter.



Text-fig. 3. A late stage of an infection through a leaf axil on a young stem.

GROWTH OF *PHOMA LAVANDULAE* IN PURE CULTURE

Phoma Lavandulae has been repeatedly isolated in pure culture from sections taken from tissues at the junctions of diseased with healthy parts of lavender stems of different ages, and also from pycnospores from pycnidia on diseased lavender bushes.

MEDIA

The first medium used was lavender agar, prepared by stiffening a strong aqueous infusion of lavender leaves with 2 per cent. agar. For initial isolations this medium was found to be most satisfactory, since in it bacterial contamination is reduced, whilst the mycelium, when isolated, has a characteristic appearance at an early stage, by which it can readily be distinguished.

Dox's agar has been used extensively. On this medium the mycelium grows more actively, and pycnidium formation can be induced whereas under no circumstances were fructifications produced on lavender agar.

The preliminary attempts to isolate the fungus were frequently unsuccessful; it was found later that many attempts were made with twigs which had the appearance of being affected with "Shab" but which in reality were suffering only from the effects of frost. Another difficulty was that in spite of careful surface sterilisation it was almost impossible to avoid contamination with *Dematium pullulans* which concealed the relatively slow growing *Phoma Lavandulae*. In later isolations, made at a time of year when frost damage was almost impossible, *Phoma Lavandulae* was successfully obtained in a high proportion of the isolations attempted. This is shown in Table I.

Table I

Source	No. of isolations attempted	No. of isolations successful
Young stems	27	19
Old stems	5	3
Inoculated plants	11	4

Although attempts were made to isolate the fungus from diseased roots none of these was successful. This is probably because the root is the last part of the plant to succumb to the disease and by the time this happens numerous secondary organisms are present which may well conceal the relatively slow growing *Phoma Lavandulae*. On the other hand, from the young stems, which are first infected, the fungus was isolated practically in pure culture from the start.

Other fungi isolated from diseased tissue were: a *Phoma* differing from *P. Lavandulae* in having larger spores, *Botrytis* spp., an un-

identified fungus characterised by the production of pycnidia containing brown spores, and a fungus allied to *Diaporthe*. Although repeated inoculations were made with all these fungi none of them proved to be pathogenic to lavender.

FRUCTIFICATION OF *PHOMA LAVANDULAE* IN PURE CULTURE

At first considerable difficulty was experienced in obtaining fructifications of *Phoma Lavandulae* in pure culture. The fungus was grown on a series of media ranging from natural substrata such as lavender twigs and potato chunks to relatively complex media such as asparagin and Dox's agar. The fungus grew on all the media tried both at room temperature and at 25° C. It was eventually discovered that if cultures on Dox's agar were kept in a room where they were exposed to bright sunshine abundant pycnidia were formed within about five weeks. Occasionally a few abnormal compound pycnidia were formed at an earlier date under these conditions, and similar pycnidia were occasionally produced in old dried up cultures even when they were not exposed to sunshine.

The *Phoma* with larger spores isolated from lavender formed pycnidia as readily in dull as in bright light.

THE GERMINATION OF PYCNOSPORES

Spores both from natural material and pure culture germinated in hanging drop cultures on films of Dox's agar with few exceptions. Within twelve hours the spores became swollen and put out one or two germ tubes (Text-fig. 4a). These grew in length, and became



Text-fig. 4. Stages in the germination of the spores of *Phoma Lavandulae*. $\times 450$.

branched and septate within a few days (Text-fig. 4*b*). From the third to the fifth days short lateral branches of limited growth were put out, and from these, groups of conidia were abstricted (Text-fig. 4*c*) which agreed very closely in size and shape with the pycnospores. The formation of similar conidia was described by Brierley.

RESISTANCE OF PYCNIDIA TO EXTERNAL CONDITIONS

Numerous experiments were made to determine the effect of certain adverse conditions on spore germination while the spores were still in the pycnidia. Freshly gathered material was kept (1) under ordinary laboratory conditions, (2) in an incubator at 25° C., and (3) in a paraffin oven at 50° C. Hanging drop cultures of pycnospores were made at intervals during a period of fourteen days. Exposure for this period to laboratory conditions or 25° C. had no effect on viability, but of those kept at 50° C. only about 50 per cent. remained viable.

No pycnidia were subjected to a known low temperature over a long period, but it was found that spores from pycnidia collected after very hard frosts or snow germinated as readily as at midsummer.

Finally, in December 1927, twigs bearing pycnidia were buried in the soil at depths of one foot and one foot six inches respectively. At intervals of one month they were dug up and the pycnospores tested. The experiment was continued until April 24th, 1928, when it was found that although the twigs were becoming decomposed the pycnospores were still as viable as at first.

EXPERIMENTS ON THE RETENTION OF VIABILITY BY SPORES AFTER EXUDATION FROM THE PYCNIDIA

The spores of many species of *Phoma* are exuded in mucilaginous drops or tendrils in a moist atmosphere. It might be expected that the spores would retain their viability so long as the mucilage is not dissolved by rain or any other agency. Attempts have been made with *Phoma Lavandulae* under different moisture conditions to induce the exudation of mucilaginous masses, but the spores of this fungus emerge from the pycnidia only when these are in contact with free water. Any mucilage that is present is immediately dissolved and the spores become separated from one another.

To determine for how long the spores of *P. Lavandulae* can retain their viability when once they have left their pycnidia, pycnospores from fresh lavender twigs were teased out in drops of sterile distilled water on sterile coverslips which were then placed in separate watch-glasses, with other watch-glasses inverted over them. The coverslips were then treated in one of the following ways:

- (1) Left at room temperature.

(2) Left at room temperature but with a drop of sterile distilled water introduced into the watch-glass so that the spores were kept in a moist atmosphere.

(3) Left at 26° C. in an incubator.

(4) Left under moist conditions as (2), but in an incubator at 26° C.

At intervals of an hour, one or two coverslips were taken from each set, and the spores covered with a film of Dox's agar at about 40° C., mounted in hanging drops and left for observation. The first experiment was continued so that the longest period of desiccation was forty-eight hours, but during this time there was no great loss of viability by the spores. Accordingly similar experiments were made at room temperature in which spores were subjected to much longer periods of desiccation, and fresh cultures were made at intervals of twenty-four hours instead of one. The results obtained may be summarised as follows:

(1) There is a general decrease in viability as the period of desiccation is prolonged.

(2) Viability is lost more rapidly in a moist than in a dry atmosphere.

(3) There is a marked loss of viability in a moist atmosphere between sixteen and forty hours.

(4) In a dry atmosphere pycnosporos retain their viability for sixty-four hours, after which they germinate more slowly than when fresh; if kept for 114 hours under these conditions only very few spores are capable of germinating.

It is thus clear that pycnidia are the chief form in which the fungus can tide over unfavourable periods, whereas isolated spores are of little importance in this connection.

INOCULATION EXPERIMENTS

Inoculations, except where specifically stated to the contrary, have been made almost exclusively on what commonly passes under the name of "Old English" lavender—a variety of *Lavandula spica* Linn. which is usually cultivated in this country for the manufacture of perfume. There is, however, considerable confusion in the nomenclature of the different varieties, and the same variety is often known by different names to different growers. What is called here "Old English" is a tall variety, with dark blue flowers arranged in interrupted spikes, usually consisting of eight to ten "whorls" of flowers. The consecutive "whorls" of flowers are sufficiently far apart for the stem to be visible between them. The heads do not taper to a point at the top. Small subsidiary flower spikes often arise in the leaf axils low down on the stem bearing the main spike.

The first inoculations were made exclusively with pure cultures of

Phoma Lavandulae, but when it was established that this fungus is the primary cause of the disease, spores from pycnidia on diseased lavender stems were employed. The preliminary inoculations were made both with mycelium and spores on wounded and unwounded plants through both young and old tissues, and both out of doors and in a greenhouse. The inoculations with spores were made with a brush or an atomiser, whilst those with mycelium were done by means of sterilised scalpels or needles. Usually where plants were inoculated at some particular point lead foil was wrapped round it and tied on securely. In addition, freshly inoculated plants were usually covered with bell jars for a period of about forty-eight hours, but this does not appear to have been essential except when plants were inoculated out of doors on dry days. Successful inoculations were also made by attaching small portions of twigs bearing pycnidia to healthy bushes.

PRELIMINARY EXPERIMENTS

Thirty-eight plants were inoculated in one or other of the ways mentioned above. Most of these inoculations were carried out in a greenhouse between October 26th, 1926 and January 21st, 1927. All the plants inoculated through freshly made wounds subsequently became diseased, but a striking feature was the long interval which elapsed between the date of inoculation and the appearance of the first disease symptoms. Thus, in a typical example, a plant inoculated with mycelium through freshly made wounds on young shoots on October 26th showed no symptoms of disease until January 12th, about two and a half months after the inoculations were made. At this date, three of the six inoculated shoots had wilted. No extension of the wilt was to be seen until the following July when the young developing shoots wilted as the fungus grew into them from the points of inoculation. By August 15th the plants were dead. When plants were inoculated with spores there was a similar long interval before the disease appeared and no rapid development took place until the young shoots succumbed very rapidly. Spore and mycelium inoculations carried out at the same time of year but out of doors, either failed, or the disease symptoms developed even more slowly than those showing in the greenhouse. For instance, a plant inoculated on October 26th remained healthy until the following June when one of the inoculated twigs wilted. By December 8th, fourteen months after inoculation, this twig was dead, but the rest of the plant was healthy.

COMPARISON OF RESULTS WITH THOSE OBTAINED BY BRIERLEY

These experiments showed conclusively that *Phoma Lavandulae* is one primary cause of "Shab" disease, thus confirming the results of Brierley. However, they differed appreciably from them in the long

lapse of time between the inoculations and the first appearance of disease symptoms. Brierley's results indicated that plants began to die back about ten days after inoculation, whereas in the present investigation a period of at least two months normally elapsed before any wilting was seen. It was thought at first that this long interval would occur only during the winter months, but this is not so. The shortest interval noted during the present investigation was in six plants growing out of doors, inoculated on June 9th, through wounded leaf axils on young shoots which became diseased one month after they were inoculated. Moreover, the period during which the disease is not apparent when plotted against the time of year when the inoculations were made takes approximately the form of an optimum curve.

Inoculations of different varieties of lavender made with strains of *Phoma Lavandulae* isolated from lavender obtained from different sources, also failed to discover any combination of a virulent strain of the fungus and markedly susceptible lavender variety which resulted in a more rapid appearance of the disease.

Another possibility was that a lavender plant growing under adverse environmental conditions might succumb to the disease more rapidly. Observations made in lavender plantations showed that the disease was usually most prevalent in low lying, badly drained areas, suggesting that excess of water at the roots favoured the disease. (The disease is by no means confined to waterlogged areas; the suggestion is merely that in such a position the plants are affected more severely. Actually the worst example of "Shab" met with was on a light, well-drained soil.)

Attempts to compare the rate of development of the disease in inoculated plants in pots kept in a bath of water with that in similarly inoculated plants in pots kept under ordinary outdoor conditions gave inconclusive results. This was because waterlogging alone is capable of causing unhealthy symptoms, and consequently it was never certain to what extent that or the fungus was individually responsible for the unhealthy symptoms noted. However, in no instance was there any evidence of the disease developing within a time that was in any way comparable with the ten days recorded by Brierley.

The only possible explanation of the long interval is that during this period the fungus is latent within the tissues of the host. It will be seen later that the latent period is longest amongst plants inoculated in autumn and winter out of doors when development appears to be retarded until the following spring. This has been borne out by microscopical examinations of tissues at varying intervals after inoculation which have shown that fungus mycelium becomes established within a few days, but that it develops very slowly until the young shoots begin to grow.

Spores of *Phoma Lavandulae* were seldom seen actually germinating

in the tissues, probably on account of their small size, but they have been met with in the pith cells near freshly inoculated wounds. They sometimes germinate several millimetres from the surface of infected wounds owing to the fact that they become embedded in cracks in the pith formed in the process of making the wound.

LARGE SCALE INOCULATION EXPERIMENTS CARRIED OUT BETWEEN OCTOBER 1927 AND SEPTEMBER 1928

During the period October 1927 to September 1928, large scale inoculation experiments were started in order to obtain information on the following points:

- (1) At what time of year infection most readily takes place.
- (2) Whether infection occurs more readily in wounded than in unwounded plants, and if so whether there is any variation in the readiness with which infection occurs according to the time of year.
- (3) To compare the time of year at which most infection takes place with that at which artificial inoculations are most successful.
- (4) To determine whether, if wounds are made at different times of the year, for how long they remain liable to infection and whether there is any variation dependent on the season at which the wounds are made.

EXPERIMENTS ON INOCULATION OF WOUNDS OF DIFFERENT AGES

It has been shown in "Silver Leaf" disease of fruit and other trees (5) that there is considerable variation in the ease with which infection takes place by spores of *Stereum purpureum* depending on the time of year at which the wounds are made, and the time that elapses between making the wounds and the introduction of the spores.

During 1926 preliminary experiments, in which lavender stems that had been wounded and left for varying periods not exceeding fourteen days and were subsequently inoculated, showed that wounds of that age were as liable to invasion as when freshly made.

Wherever it is grown on a commercial scale lavender is liable to be severely wounded (1) when the crop of flowers is cut in August, (2) in September or October when some growers make a practice of trimming the plants with shears, (3) early in March when the plants may be trimmed with shears—a practice suggested as a means of keeping the disease in check. Consequently in the following experiment wounds were made in August, October and February respectively, and inoculated at successive intervals of one month to determine whether such wounds were equally liable to invasion, and if so, for how long they were likely to remain so.

A. August wounds

Seventy-two plants were divided into groups of three so that one set could be inoculated each month throughout the year, and a similar set could be kept as a control. Three plants, wounded on August 4th by cutting off pieces of the younger branches with scissors, were immediately inoculated with a spore suspension of *Phoma Lavandulae*, and successive sets were inoculated on August 28th, October 18th, November 11th, December 9th, January 18th, February 6th, March 14th, April 4th and May 26th respectively. Each wound was covered with a cap of lead foil tied on with wool immediately after it had been inoculated.

All three of the plants inoculated on August 4th, when the wounds were fresh became diseased, the first symptoms being noted on November 12th. During the following summer the three plants became badly diseased and by August 11th one was nearly dead. This corroborated the conclusion already reached, viz. that infection can occur through freshly made wounds.

The plants inoculated on August 28th, when the wounds were about three weeks old, showed by December 12th rather uncertain disease symptoms which underwent no further development and were confined to one shoot.

Up to October 1928 none of the plants inoculated after August 28th became diseased and the controls likewise remained healthy. For this reason these inoculations were discontinued after May 26th.

B. October wounds

The wounds made in October did not become infected even when they were freshly made, probably because owing to the very small size of the plants it was almost impossible to make wounds in living tissues without cutting most of the plant away. In consequence the wounds were made in young twigs which were already dying back naturally at the end of the summer as is customary in all lavender. Other experiments described later show that wounds made in October are actually liable to infection when fresh.

C. February wounds

A third set of plants were wounded on February 28th, 1928. Six of these were inoculated on March 4th, when the wounds were five days old, and further groups of six were inoculated at intervals of approximately one month until June. Six uninoculated plants were marked as controls on each occasion.

Of the plants that were inoculated through wounds five days old, two showed early disease symptoms on July 17th and by August 7th four were diseased. By November 17th, two of the plants were

continuing to die back slowly and two others were nearly dead. The remaining two plants kept free from disease as did also the control plants except one that was killed by frost.

Six plants inoculated on April 25th through wounds that were three and a half weeks old remained healthy. Of six plants inoculated on May 26th one was dying back rapidly on November 17th, and of those inoculated on June 16th, when the wounds were three and a half months old, two were badly diseased by November 17th and one less severely. The control plants for each month remained healthy. It thus appears as if wounds made in March may remain liable to infection for several months, but this is probably not really so, the results depending on the fact that from May onwards the plants were growing rapidly and that consequently in attempting to gain access to the wounds in order to inoculate them fresh wounds were made unintentionally through which infection probably took place. If this explanation is correct the conclusion may be drawn that fresh wounds made in March are liable to be invaded by *Phoma Lavandulae*, in the same way as those made in August and October, and that in all three months this liability to invasion is lost after an interval of four weeks.

MONTHLY INOCULATION EXPERIMENT

Starting in October 1927, three sets of six plants each were treated at intervals of approximately one month as follows: (a) six plants were wounded by trimming them with scissors, and sprayed with a spore suspension of *Phoma Lavandulae* immediately afterwards; (b) six unwounded plants were sprayed in the same way; (c) six unwounded plants were sprayed with sterile distilled water and left as controls.

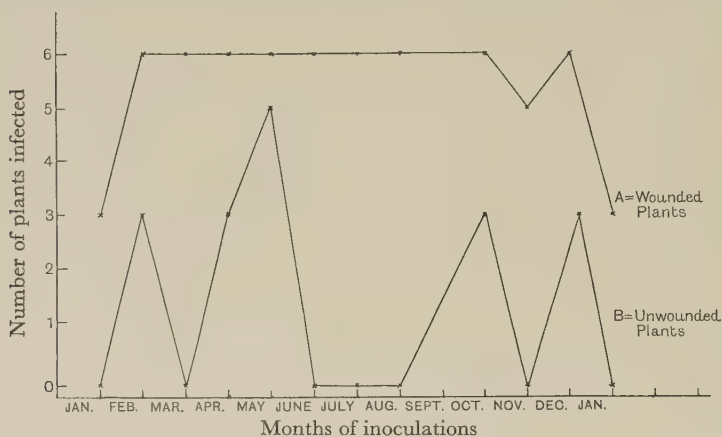
Immediately after inoculation these were covered with bell jars for a period of about forty-eight hours, or longer during some of the winter months. No bell jars were placed over the control plants as there were not sufficient available for the purpose.

Up to September 30th, 1928, no control plant showed any disease symptoms. However, by November 17th, 1928, eight control plants had become diseased—a result which is scarcely surprising since by this date the disease was rampant amongst the adjoining plants that had been inoculated.

The result of the experiment is shown in the form of a graph in Text-fig. 5. Certain important points have been made clear. Thus it is shown that infection can take place, under suitable conditions, during any month. The lowest infection recorded was amongst the plants inoculated in January, of which only two that were freshly wounded developed the disease, and none that was unwounded. From February until May there is a steady increase in liability to infection both amongst wounded and unwounded plants, and finally in May, six wounded and five unwounded succumbed. From May onwards

during the summer, whereas all the freshly wounded plants that were inoculated became diseased the unwounded ones remained healthy. It is of interest that the maximum infection of unwounded plants was in May, when the young shoots were developing, which agrees well with what was observed in the field. When the young shoots become hardened as they develop they become less liable to invasion unless they are wounded.

Another important feature is the long interval that elapses between the time of inoculation and the first appearance of disease symptoms. Thus, when plants were inoculated at the beginning and middle of November respectively, a period of seven months elapsed before the



Text-fig. 5. *A* = number of infections during successive months in wounded plants.
B = number of unwounded plants successfully inoculated during successive months.

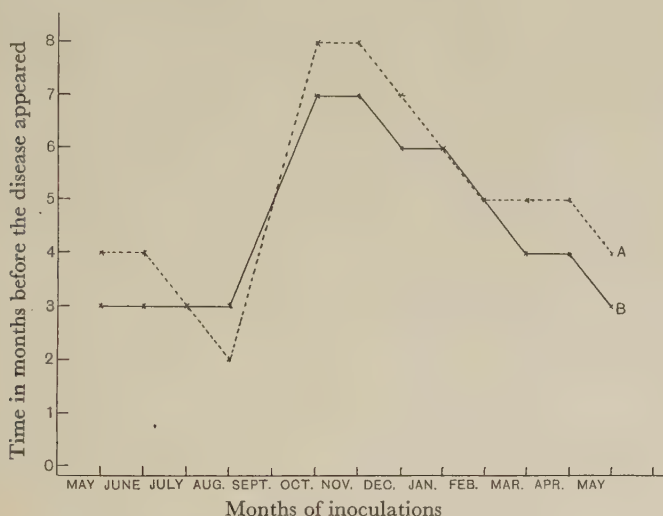
disease could be discerned. This period gradually diminished amongst the plants inoculated during consecutive months until May to August, when there was an interval of three months only. These results are expressed graphically in Text-fig. 6.

The successful inoculations of October, which took more readily in wounded than in unwounded plants, show that freshly made wounds are liable to invasion at that time of year. This is contrary to the results obtained in the experiment dealing with the inoculation of wounds of different ages, and supports the suggestion there put forward to account for the failure then experienced (p. 161).

EXPERIMENTAL INOCULATION TRIAL BY ATTACHING TWIGS BEARING PYCNIDIA TO PLANTS AT MONTHLY INTERVALS THROUGHOUT THE YEAR

Starting in early November 1927, small portions of diseased lavender stems bearing pycnidia of *Phoma Lavandulae* were attached to the upper branches of healthy plants by means of fine copper wire.

Twenty-five plants (sometimes twenty-four) were subjected to this treatment each month. After the first month the pycnidia-bearing twigs were removed from twelve of the plants to which they had been attached one month previously. In this way the plants were exposed to a source of infection for long and short periods respectively, starting at different times of the year. Each month twenty-five plants were left untreated as controls, and during the course of the experiment only four out of about three hundred became infected.



Text-fig. 6. *A* = time during which the fungus is latent within the host amongst plants inoculated by attaching twigs bearing pycnidia to them at intervals of one month.
B = ditto in plants inoculated with spores.

The detailed results are shown in Table II. The most interesting points brought out are (1) that infection can occur at any time of year; (2) that, generally speaking, exposure to a source of infection for a prolonged period is more effective than exposures of not more than four weeks; (3) that there is a period ranging from two to nine months during which the fungus is dormant within the host tissues according to the time of year when the inoculations are made. The duration of this latent period is in very close agreement with that found when inoculations with spores were carried out at monthly intervals as described in a previous experiment. This is brought out very clearly in Text-fig. 6 in which the duration of the latent period in these two experiments is seen to approximate very closely.

INOCULATIONS OF DIFFERENT VARIETIES OF LAVENDER AND OTHER PLANTS

Experiments in which as many different varieties of lavender as could be obtained were inoculated with *Phoma Lavandulae*, both out of doors and in a greenhouse, have led to the conclusion that most varieties are about equally susceptible to invasion. A notable exception is the variety "Dwarf French" which failed to become diseased under any circumstances, and indeed, it seems probable that this variety is immune from the disease. The oil obtained by the distillation of the flowers of this variety was very promising when freshly distilled, but was not suitable for lavender water.

Attempts have also been made to inoculate *Lavandula dentata*, *Rosmarinus officinalis*, *Lamium purpureum*, and *Chenopodium album*, all of which were unsuccessful.

Table II

Date of inoculation	Long or short exposure	Time in months before disease appeared	No. of diseased plants in consecutive months				Killed by frost
			June	July	August	Sept.	
Nov. 2nd	L.	8	6	7	8	8	1
	S.	9	0	1	1	3	4
Nov. 18th	L.	8	4	5	6	6	3
	S.	8	1	2	2	3	1
Dec. 12th	L.	7	5	7	9	9	2
	S.	8	0	7	7	8	2
Jan. 14th	L.	6	2	8	8	9	1
	S.	7	0	2	2	3	1
Feb. 8th	L.	5	4	7	9	10	—
	S.	5	1	7	7	8	1
Mar. 15th	L.	5	0	6	8	10	—
	S.	5	0	3	3	7	3
Apr. 23rd	L.	5	0	0	1	4	4
	S.	5	0	0	1	3	4
May 25th	L.	4	0	0	1	6	1
	S.	3	0	0	1	6	1
June 12th	L.	4	0	0	0	2	—
	S.	4	0	0	0	5	1
July 17th	L.	3	—	0	0	3	—
	S.	No disease	—	0	0	0	—

FURTHER OBSERVATIONS ON THE MODE OF SPREAD OF THE DISEASE

A feature of the disease that is frequently very striking is that it is often confined to localised areas in the field. This has been thought in the past to indicate that some soil factor played an important part in the causation of the disease, lime deficiency especially having been held responsible. During the present investigations it has been noted that the trouble is often most severe in low lying places where water tends to accumulate in winter, and there is no doubt that excess of water at the roots of itself can induce unhealthy symptoms in the crop,

and in conjunction with *Phoma Lavandulae* the disease would have the appearance of being especially well developed in waterlogged areas. The disease has also been seen on soils throughout which there is known to have been a good lime content, so that lime deficiency cannot always be held responsible. The worst case of "Shab" met with was on a light, well drained, calcareous soil, but here also localised disease centres were met with, showing that the distribution must be accounted for in some other way.

Detailed observation extending over several years made in a number of lavender plantations have led to the conclusion that the uneven distribution of the disease exists only at an early stage before the fungus has become widely distributed. The areas in fact represent the random positions in which the first diseased plants were established and from these infection spreads in a centrifugal manner, resulting first of all in the formation of disease centres which later on coalesce and lose their individuality. This mode of spread accords very well with what would be expected from the manner in which the fungus is dispersed. The pycnosporos are very small and thin walled. Moreover, when once they have been exuded from the pycnidia they cannot remain long alive. Such spores are clearly not well adapted for wide dispersal by wind. At the same time the spores are incapable of emerging from the pycnidia except when the latter are in contact with water. When it rains heavily, newly exuded spores are splashed about and so spread over short distances. After a heavy storm it has been noted that soil particles are frequently splashed to the top of large lavender plants, and if it be assumed that spores may be splashed to the same height it is clear that they may in this way gain access to leaf axils on young developing shoots and so establish infection. This mode of spore dispersal probably accounts for the centrifugal extension of the disease.

Sometimes a single plant, or two or three in a group at some distance from any diseased plants, will themselves become infected, and observations have been made to determine whether the pycnidia themselves rather than the spores can become disseminated over wider areas, and considerable evidence has been found in support of this suggestion.

Cultivation in lavender plantations is normally carried on by horse-hoeing and it is largely as a result of this practice that the fungus is distributed over wide areas. As the hoe passes between the rows of plants small fragments become detached. The young pycnidia-bearing twigs of diseased plants being very brittle are especially liable to be broken off, and after the hoe and the driver have passed by there is an elastic rebound of the bush which results in hurling into the air fragments which fall on or near healthy bushes in the neighbourhood of the diseased ones. In addition, twigs bearing pycnidia may be

carried a considerable distance attached to the horse's hoofs, the hoe, and the driver's boots.

Further, it is common, especially during the winter, for the outermost tissues to break away from young twigs, and to fall off subsequently in small particles, and if the twig is a diseased one the pycnidia sometimes come off also. Experiments were made to determine whether these pycnidia-bearing particles become distributed by the wind. This was done by exposing to the wind pieces of American cloth coated with vaseline, in a field near diseased bushes. The cloth was held in a more or less vertical position by means of a wooden framework. Small particles of all kinds adhered to the surface of the cloth which was subsequently examined. After examination, the cloth was cleaned with xylol, recoated with vaseline and used for repeated exposures. Most of the experiments were unsuccessful, but on a few occasions small particles of lavender bark bearing pycnidia of *Phoma Lavandulae* were caught and identified under the microscope. These particles were caught at a distance of about two feet from a diseased plant. It is therefore considered that pycnidia may be distributed in this way at least for short distances, although probably to a somewhat limited extent.

FACTORS OTHER THAN *PHOMA LAVANDULAE* WHICH MAY CAUSE SYMPTOMS SIMILAR TO "SHAB"

The symptoms of "Shab," especially at an advanced stage, are such that any factors which adversely affect the lavender plant would simulate those resulting from invasion by *Phoma Lavandulae*. It is believed that this does happen, and the death of lavender resulting from such causes has undoubtedly led to confusion in the past.

The most important of these adverse factors are thought to be frost, shading by or dripping from trees, and possibly invasion by a species of *Botrytis* either alone or in conjunction with the other factors.

Frost can damage lavender plants in either of two ways. A severe frost in winter may cause large branches to die back or even result in the death of the plant. Newly established plants are especially liable to this type of injury, as are also those that have been clipped severely in late summer or autumn. On the other hand less severe frosts in late spring may cause the young shoots to turn yellow and subsequently to die back. This type of damage might readily be mistaken for an early stage of *Phoma* attack. Indeed, the damage resulting from these two causes was finally distinguished only by making detailed periodical observations on a large number of twigs. Generally speaking, the yellowing due to the late frosts appears earlier in the season than the similar symptoms induced by *Phoma*. Injury from late frosts is seldom serious as fresh shoots develop which replace the damaged

ones, and the worst that may happen is that the time of flowering may be somewhat delayed.

Lavender plants have sometimes been seen showing symptoms similar to those of "Shab" but the cause is uncertain. Large branches die, and their foliage sometimes turns yellow before they succumb. Infected branches become covered with a *Botrytis*, and hyphae, which may belong to the same fungus, are found in the tissues. In the absence of successful attempts to inoculate lavender with the *Botrytis* this cannot be held responsible for the disease, but it is possible that it may act as a weak parasite in plants that have been previously weakened by some such cause as frost.

Finally it may be pointed out that in old lavender bushes, as in other woody plants, a certain amount of die back appears to be normal even when they are growing under ideal circumstances.

OCCURRENCE OF *PHOMA LAVANDULAE* ON *CHENOPODIUM ALBUM*

While searching on the ground in a lavender field for old diseased stems bearing pycnidia of *Phoma Lavandulae*, a number of pycnidial fungi were seen on decaying weeds. Among these a *Phoma* morphologically indistinguishable from that on lavender was found on decaying stems of *Chenopodium album*, a common weed in most lavender fields, and extremely abundant in some. Further collections of decaying *Chenopodium album* were examined on which the fungus was found to be quite common, especially in those parts of the field in which the lavender disease was most abundant. Careful search was made for the fungus on other weeds, but without success.

The fungus was isolated and grown in pure culture and agreed in its growth characters with *Phoma Lavandulae* and, moreover like it, could be induced to fructify only in a bright light. The fungus has been isolated from *Chenopodium album* several times during two successive seasons, but only from one field. However, no serious search for the *Phoma* on *Chenopodium album* was made in any other lavender plantations.

Inoculations of lavender plants were made in a greenhouse with mycelium and spores. Seven plants in all were inoculated, four of which developed typical disease, two being killed; the remaining three plants remained healthy while under observation. The *Chenopodium* fungus thus appears to be identical with *Phoma Lavandulae*.

Phoma Lavandulae has not been found on living plants of *Chenopodium album*, nor have inoculations of living specimens met with any success.

Another species of *Phoma* has been found on living and dead plants of *Chenopodium album*, but this fungus has larger spores and smaller pycnidia than *Phoma Lavandulae*. Furthermore, its growth form in pure culture is quite distinct. On dead stems of *Chenopodium album* the

pycnidia of both *Phoma* species have been seen growing together, but generally speaking *Phoma Lavandulae* has been found on *Chenopodium album* only when the latter was in a fairly advanced stage of decomposition, whereas the large spored *Phoma* tends to disappear at that stage. No evidence of a genetical connection between the two species has been established. Inoculations of lavender plants with the large spored *Phoma* were unsuccessful.

CONTROL MEASURES

Since the only variety of lavender found to be immune to the "Shab" disease is of no use in the manufacture of perfume, the disease cannot be controlled by growing resistant varieties. From the nature of the disease no satisfactory cure can be effected, and it is best controlled by general methods of sanitation. Attempts to control the disease along the lines described below have already been introduced into some plantations and show considerable promise, and if continued may lead to the complete extermination of the disease.

(1) *Change of land*

Lavender has been cultivated in this country on a commercial scale in strictly localised areas, chiefly by growers who confine themselves almost exclusively to this one crop. With the natural desire to obtain the maximum yield from the land, lavender crops have been grown continuously on the same ground for seventy years or longer, and crop rotation has been almost unknown. This is undoubtedly the chief reason why the "Shab" disease has become so firmly established.

It has been shown that dead branches bearing pycnidia become detached from diseased lavender bushes, and may remain on the ground until they undergo decomposition. Hence, if the ground in a diseased field is replanted with healthy bushes immediately after the previous crop is removed the fresh plants will be subjected to a source of intense infection. Further, successive crops on the same ground will become progressively worse, and, indeed, the ground would soon become lavender "sick" and unfit for the cultivation of this crop until it had had a rest. It is strongly urged that before any area is replanted with lavender, whether the disease is present or not, it should have a change of crop for at least one year, or if necessary it should be left fallow.

Lavender cultivation on a heavy, waterlogged soil should be avoided. If this is impossible all measures conducive to good drainage and "lightening" the soil should be practised.

(2) *Clean cultivation*

Lavender plantations should be kept in clean cultivation. In recent years, however, weeds have become all too common in the plantations

in some districts, rendering the fields unsightly and resulting in a great weakening of the plants. Hand hoeing is the ideal method of keeping a lavender plantation clean, and even in large areas where horse-hoeing is the only commercial possibility weeds immediately surrounding the plants must be removed by hand. Neglect of this last precaution has been the cause of severe epidemics of weeds in some districts, resulting in a great weakening of the bushes which were rapidly overcome by the fungus as a result.

Special care should be taken to remove all plants of *Chenopodium album*, the dead remains of which may become infected with *Phoma Lavandulae*, and so assist in carrying over the fungus to a more favourable period for its development. This weed may also serve as a means of keeping the fungus alive between two successive crops.

(3) Taking cuttings

In the past the usual methods by which lavender has been propagated are: (1) By dividing a large plant into a number of small ones with the aid of a chopper, in such a way that each of the fresh plants is provided with a portion of the root system of the parent. (2) By breaking off branches at least two or three years old, and planting them deeply in the soil where they develop roots and grow into fresh plants. (3) By burying plants in the soil to such a depth that only the young branches are above ground. Adventitious roots grow out from all parts below the soil, and when these are well developed the original plant is dug up and divided into a number of small ones. This method of propagation is common in nurseries.

All these methods are unsatisfactory where the "Shab" disease is present, and should be discontinued, because by any of them the mycelium of *Phoma Lavandulae* may be distributed in a high percentage of the young plants. Even if care is taken to avoid propagating from diseased bushes this may, nevertheless, be done quite inadvertently, since the fungus may be established in a latent condition in an apparently healthy plant.

If possible a fresh start should be made with a healthy stock planted in clean ground. Clean stocks of good oil-yielding varieties are not easy to obtain, however; hence growers who are unable to start afresh may propagate their plants in the following way.

Small cuttings are less liable to be diseased than large ones. It is, therefore, suggested that small cuttings should be used exclusively for propagation. The cuttings should be only two to two and a half inches long and should consist only of "green wood." It has been found that the cuttings root most readily if started in a light soil in a warm greenhouse, but if this is not practicable they may be grown in cold frames or even in open ground in a sheltered place, but here growth is slower. One grower has taken many thousands of cuttings

in this way from a badly diseased stock with most promising results, a small percentage only of the cuttings becoming diseased. However, the diseased plants were destroyed as soon as they appeared, and one year after the cuttings were taken many thousands of strong young plants were obtained which were planted out in their permanent quarters.

When propagating lavender in this way allowance must be made for the fact that some of the cuttings fail to root. If reasonable care is taken the percentage is not a high one, but it is always advisable to take a few thousand more cuttings than the number required.

(4) *General sanitation*

As soon as a diseased plant is seen in an otherwise healthy plantation it should be destroyed without delay. The four plants nearest to it should also be destroyed, since the fungus may be established in them in a latent condition.

(5) *Distance between the plants*

When lavender was first cultivated in this country it was customary to plant the bushes further apart than the three feet or less which is the present custom. As a result of inspecting plantations in which the bushes were at different distances apart, it has become clear that the greater these distances are, so much the more do the plants flourish. An excessive distance between the plants is clearly not economical as a great deal of ground is wasted, but a distance apart that has much to commend it is four feet in each direction. In a young plantation this allows room for the horse-hoe to move diagonally through the field, as well as in directions at right angles to one another. Furthermore, since the branches of neighbouring plants do not grow in contact with one another, spores are not so liable to be splashed from plant to plant.

(6) *Spring clipping*

A promising means of controlling the disease is to clip the plants drastically with shears at the end of February or the beginning of March. This is based on the consideration of the fact that the fungus develops slowly during the winter. Furthermore, since the plants are wounded most severely when the crop of flowers is harvested in August, and in some plantations additional wounds are made by trimming the plants with shears in the autumn, it is considered that the plants are especially liable to infection immediately after these operations. Moreover, since the fungus develops only slowly during the winter months it was hoped that if the plants were trimmed drastically with shears in the spring most of the newly infected portions would be removed, and in consequence a clean start would be made

during the summer. This method of control would be ineffective in a plantation that is already badly diseased, or if the clippings are not carefully collected and burned as soon as the trimming has been carried out. When the experiments to test this idea were started it was hoped that wounds made in the spring would not be so liable to infection as those made in August or September, but this possibility was later conclusively disproved by the inoculation experiments already described.

Apart from any consideration of the disease the practice of spring clipping renders the plants more liable to be damaged by late frosts in May, when the young shoots turn yellow and later die back. However, they recover from this injury and the main consequence is that the time of flowering may be delayed by about three weeks. This delay is a serious drawback in many instances, and where early flowering is important the practice is not recommended.

The first important experiment was started on March 19th, when four rows, each consisting of about seventy plants, were trimmed drastically with shears. Four similar rows alternating with the clipped ones were left untreated as controls. No disease was apparent in any of the plants before clipping. The results are shown in Table III.

Table III

Date	No. of diseased plants in clipped rows	No. of diseased plants in unclipped rows
July 6th	4	20
July 19th	5	24
August 2nd	11	37

In the autumn of 1927 the diseased plants in six of the eight rows were removed, and the plants in three of them trimmed again on March 6th, 1928, the behaviour of the bushes thus being kept under observation for two consecutive seasons. The results are shown in Table IV.

Table IV

Date	No. of diseased plants in clipped rows	No. of diseased plants in unclipped rows
March 5th	5	20
May 21st	1	20
June 14th	13	49
July 17th	30	85
August 5th	41	105

In another experiment, twelve groups of six rows of four-year old plants each extending the whole width of a field were treated as follows. Two rows were trimmed drastically with shears on August 20th-26th, 1927, two were trimmed on March 5th-6th, 1928, and the

remaining two were left untreated as controls. This arrangement was adopted because the disease was principally situated in localised areas. Most of the badly diseased bushes were dug up and destroyed at the end of August 1927, but those that were only slightly affected were left.

By November 1927, a great many branches in the plants clipped between August 20th and 26th had died, and many more succumbed during the winter, chiefly following intense frost or heavy rain. Finally, by the spring of 1928 most of these plants were dead, and during the summer only a few of the remainder recovered sufficiently to produce flowers. The wood of the moribund plants was brown, and infested with fungal mycelium. Attempts to isolate the fungus in pure culture were made, but only *Dematium pullulans* and other common saprophytes were obtained.

The remaining plants were studied individually. The first record was made immediately before the spring clipping, those plants on which pycnidia of *Phoma Lavandulae* were present being marked as diseased. The number of plants thus kept under observations was 3443, of which 1723 were clipped and 1720 were kept as controls. There were 435 diseased plants before clipping, and 1747 were diseased on August 5th, when the last observations were made—an increase of 1312 during the summer. Furthermore, comparison shows that whereas there was an increase of 445 diseased plants in the clipped rows the increase in those that were unclipped was 887. The incidence of the disease in clipped and unclipped rows during successive months is shown in Table V.

Table V

Date	Diseased plants in clipped rows	Diseased plants in unclipped rows	Total change in clipped rows	Total change in unclipped rows
8. iii. 28	219	216	—	—
17. v. 28	77	239	Decrease of 142	Increase of 23
12. vi. 28	173	595	Decrease of 46	Increase of 379
9. vii. 28	468	903	Increase of 249	Increase of 687
2. viii. 28	644	1103	Increase of 425	Increase of 887

The increase in the number of diseased bushes in clipped and unclipped rows respectively during successive months is shown in Table VI

These figures show conclusively that spring clipping may lead to a very marked reduction in the spread of the disease. Moreover, it is considered probable that if the practice is carried out regularly the

result would be even more satisfactory, since clipped and unclipped rows would not then be intermingled as they were in this experiment. Most of the later infections in the clipped rows were undoubtedly due to the close proximity of the pycnidia in the control rows, for which reason the small units used were unsatisfactory.

Table VI

Month	Change in clipped rows	Change in unclipped rows
March 31st—	decrease of 142	increase of 23
May 17th—		
May 17th—	increase of 96	„ 356
June 12th—		
June 12th—	„ 315	„ 356
July 9th—		
July 9th—	„ 176	„ 200
August 2nd		

It will be seen from the figures in Table V that to begin with there was actually an apparent decrease in the number of diseased plants in the clipped rows immediately after the clipping had been carried out, which might be thought to indicate that certain of the plants were "cured." Although this may have been so in certain instances, the effect in others may have been only an apparent one due to the fact that all those parts of the plants that were obviously diseased were removed; but the fungus may have been established below the points of abscission and have undergone further development afterwards, the plants thus becoming obviously diseased a second time. On the other hand a bush that was only slightly diseased may well have been "cured," and it is almost certain that many such "cures" were masked by the plants becoming infected a second time after being trimmed.

Further experiments on the same lines were conducted in another field about one and a half acres in extent. No disease was present in this field, and the plants were young, one acre being planted with second year bushes and the remainder were in their first year. Although no disease appeared in this plantation certain interesting features were noted, of which the most important was that the time of flowering was not delayed by spring clipping. Indeed, very little difference could be seen between the plants that were clipped in spring and those that were not trimmed at all—a feature which indicates that if spring clipping is started when the plants are sufficiently young the drawback of late flowering may be avoided. The second interesting result shown by this experiment is that a high percentage of the plants clipped in late summer (September 6th) were killed by frost, thus corroborating the result obtained in the previous similar experiment with older bushes.

Observations were also made in a small commercial lavender plantation where drastic spring clipping had been practised regularly

for many years. This plantation contained no diseased plants. Furthermore, the time of flowering was not seriously delayed, the plants displayed strong and vigorous growth, and the flower heads were remarkably fine. All these facts tend to show that in competent hands the practice of spring clipping is advantageous.

OTHER DISEASES OF LAVENDER

Two other diseases of lavender have been met with that are distinct from "Shab." These diseases do not cause much damage to the crop and are of no economic importance. No detailed work has been done on either of them.

A. Circular black spots a few millimetres in diameter are formed on the leaves, often in great numbers. The centres of the spots sometimes become white as the tissues are killed, and pycnidia of *Septoria Lavandulae* are formed on the spots. Usually the older leaves are attacked. If the attack is a severe one some of the leaves fall off prematurely, but otherwise very little harm results.

B. The stems bearing the flower heads frequently become constricted and twisted usually an inch or two below the actual flowers. Later the tissues at the lesions become partially decomposed and the heads then fall over. The lesions never extend very far down the stems, seldom attaining a length of more than a few centimetres. The diseased tissues are frequently packed with fungal mycelium, and in damp weather a species of *Botrytis* fructifies over the surface of the lesions. The cause of this disease, which is most prevalent in old bushes, especially if the weather is wet, is unknown.

DISCUSSION OF RESULTS

"Shab" disease is a very serious one and if lavender had been cultivated in this country on a larger scale it would undoubtedly have commanded more attention at an earlier date. The species of *Phoma* which are of economic importance are chiefly associated with rots, such as "dry-rot" of swedes caused by *Phoma Lingam*. *Phoma Lavandulae* is of especial interest in that it is capable of growing throughout the tissues of a lavender plant in which it can induce a wilt and die back—a mode of parasitism unlike that so far recorded for any other member of the genus.

There are very few plant diseases in which inoculations have been made at monthly intervals throughout the year. This, in the main, is doubtless due to the fact that many agricultural crops are comparatively short lived and hence do not lend themselves to this type of experiment. However, in recent years very extensive work on these lines has been carried out in connection with the "Silver Leaf" disease of fruit and other deciduous trees by Brooks and his colleagues. In

lavender large deposits of gum are not formed in association with wounds, and in correlation with this fact it has been shown that fresh wounds in lavender stems are liable to invasion at any time of the year under suitable conditions. It is true that old wounds in lavender stems cannot be invaded by *Phoma Lavandulae*, in the same way as old wounds in plum wood are not liable to invasion by *Stereum purpureum*; but whereas in plum wood this is usually due to the deposition of gum, in lavender it results from some other as yet undetermined cause.

The factors governing the fructification in pure culture of members of the Sphaeropsidales is little understood. It has been found in a general way that such factors as light intensity and temperature play a part in this connection, but no general rules applicable to more than a few isolated species within the group have been discovered (8, 9). The problem of pycnidium formation is a complex one, which, if more fully understood, would greatly assist plant pathologists.

The discovery of *Phoma Lavandulae* growing saprophytically on *Chenopodium album* indicates the possibility that as yet the biology of fungi of the *Phoma* type is but little understood. Members of the Sphaeropsidales have hitherto been studied chiefly from the standpoint of the crop plants on which they are known to be parasitic, and it is sometimes assumed that fungi of this type are relatively specialised parasites. *Phoma Lavandulae* will not grow on *Lavandula dentata* and other Labiatae, nor even on the "Dwarf French" variety of *Lavandula spica*, although it will grow saprophytically on *Chenopodium album*.

SUMMARY

1. It has been shown conclusively by inoculation experiments that the primary cause of the "Shab" disease of lavender is the fungus *Phoma Lavandulae* Gab. Symptoms similar to those of "Shab" may, however, be due to adverse climatic and environmental factors of which the chief are frost and waterlogged soils. A species of *Botrytis* may also cause a die-back at times.

2. The early symptoms of the disease are that the young shoots turn yellow and wilt from about May onwards. Later the branches die back, and the disease spreads throughout the plant which ultimately dies. Pycnidia of *Phoma Lavandulae* are formed in great numbers on the young diseased shoots after these have been killed.

3. Infection of healthy lavender plants can take place either through or near leaf axils on young shoots in summer, or through freshly made wounds. From the points of infection the fungus grows downwards invading all the tissues, and killing the branches. Wounds are liable to invasion when freshly made at any time of the year under suitable conditions, but when more than four weeks old they appear no longer to serve as paths of infection.

4. A long period elapses between the time when a plant is inoculated and the first appearance of clear disease symptoms. During this period, which ranges from two months to a year or more, the fungus is latent within the tissues of the host.

5. The disease is spread (1) by raising plants from cuttings in which the fungus is already established, (2) by the distribution during cultural operations of branches or leaves bearing pycnidia, (3) by the distribution by wind of small particles of bark bearing pycnidia, and (4) locally by the splashing of spores from plant to plant by rain.

6. *Phoma Lavandulae* has been isolated repeatedly from diseased lavender plants and grown in pure culture on a variety of media. It failed to produce pycnidia except on a few media of which the chief was Dox's agar in bright sunlight.

7. Pycnosporos of *Phoma Lavandulae* lose their viability within a few hours of being exuded from the pycnidia. On the other hand, they can remain alive within the pycnidia for long periods under a wide range of environmental conditions.

8. Whilst *Phoma Lavandulae* can infect most varieties of lavender the variety "Dwarf French" appears to be immune. This variety is of no use in the manufacture of perfume. *Lavandula dentata* is likewise immune. On the other hand, *Phoma Lavandulae* can grow saprophytically under field conditions on *Chenopodium album*.

9. Measures for controlling the disease based on the principles of plant sanitation have been devised and have met with considerable success wherever they have been put into operation.

DESCRIPTION OF PLATE VII

Fig. 1. A freshly wilted lavender twig showing its attachment to a branch that is still healthy. $\times \frac{1}{2}$.

Fig. 2. A group of lavender plants that were killed by "Shab" disease, with a background of healthy bushes of the same age.

BIBLIOGRAPHY

- (1) BRIERLEY, W. B. "A Phoma disease of lavender." *Kew Bull.* (1916), 113-131.
- (2) CHEVALIER, A. "Sur une maladie de la lavande cultivée." *Rev. Bot. appliquée* (1922), 482-483.
- (3) LAZARE, A. "Le pourridié de la lavande." *Parfumerie moderne*, xvii (1924), 62. (Abstract in *Bull. Soc. Bot. Fr.* LXXII, sér. v (1925), 286.)
- (4) WHITEHEAD, T. and PRITCHARD JONES, W. A. "'Dry-rot' of swedes." *Welsh Journ. Agric.* v (1929), 159-175.
- (5) BROOKS, F. T. and MOORE, W. C. "Silver leaf disease. V." *Journ. Pom. Hort. Sci.* v (1926), 61-97.
- (6) — *Plant Diseases.* Oxford (1929).
- (7) SWARBRICK, T. "The healing of wounds in woody stems." *Journ. Pom. Hort. Sci.* v (1926), 98-114.
- (8) LEONIAN, H. "A study of factors promoting pycnidium formation in some *Sphaeropsidales*." *Amer. Journ. Bot.* xi (1924), 19-50.
- (9) MOHENDRA, K. R. "A study of changes undergone by certain fungi in artificial culture." *Ann. Bot.* XLII (1928), 863-887.



Fig. 2.



Fig. 1.

PUCCINIA MALVACEARUM IN MONOSPORIDIAL CULTURE

By DOROTHY ASHWORTH

Royal Holloway College

(With Plates VIII and IX and 7 Text-figures)

DURING recent years the attention of botanists has again been drawn to the rusts by the suggestive results of Craigie's experiments with *Puccinia Helianthi* and *P. graminis*. These results, stimulating and suggestive though they be, call for more precise experiments, and there seems need for single spore cultures made from the uninucleate spores—pycnidiospores or sporidia. The present investigation was undertaken in an attempt to supply this need. The material used was determined chiefly by the fact that in the botanical garden of Royal Holloway College there appeared to be a continuous supply of teleutospores of *Puccinia Malvacearum* which have the convenient character of germinating without a period of rest, so that sporidia can be obtained in great numbers at any time of the year. This is a "short cycled" rust and there are no pycnidiospores in the life-cycle. Sporidia of *Puccinia Malvacearum* were therefore chosen for single spore cultures. They are very small and very delicate, but with a Chambers's micro-manipulator it has been found possible to transfer them, without exposure to desiccation, on to a host. The host plants, species of *Malva* and *Althaea*, which were used for the inoculation experiments, were grown from seed and kept in a greenhouse, far removed from the garden where the naturally infected plants were growing.

It is apparent from the literature on this fungus that there are several gaps in our knowledge of the life cycle, and also several points upon which further evidence is desirable.

In England the disease has been studied more from an economic than from a purely scientific standpoint. As early as 1882 Plowright⁽²⁷⁾ considered the general facts of the life-cycle. A sudden epidemic of the hollyhock disease in Manchester led Robinson⁽²⁸⁾ to study the relations of the fungus to the host plant, the pathology of the invaded cells, and the extent of invasion of the tissues.

In America, Taubenhaus⁽³¹⁾, Dandeno⁽¹⁶⁾ and Duggar⁽¹⁷⁾ have all made contributions. The first interested himself in the problem of overwintering of the fungus, and is opposed to some of the suggestions brought forward by the other two American writers on this point.

Perhaps the name most readily associated with *Puccinia Malvacearum* is that of Eriksson^(18, 19), who claims to solve the hibernation problem by his mycoplasma theory. He considers evidence, culled from the behaviour of *Puccinia Malvacearum*, to be in complete agree-

ment with his mycoplasma hypothesis. In England this work has been most convincingly refuted by Bailey (2).

The observations on overwintering are so confusing and contradictory, that it was thought probable that the rust might behave differently in different climates. That it is affected by external conditions is evident from the fact that Buller finds it difficult to cultivate in Winnipeg. It therefore seems possible that the difference in behaviour reported by Dandeno and Eriksson may be explained by the difference in climate. In this study the fungus has been found to be most dependent on suitable moisture relations, and the non-occurrence of these might lead to some slight difference in behaviour. So far no one in England has observed the manner in which the fungus passes the winter, and experiments were therefore made to investigate this.

Puccinia Malvacearum is one of the rusts which has been studied to find the mode of origin of the diplophase in short cycled forms. From comparison with events in the aecidium of *Phragmidium*, Blackman and Fraser (6) suggested a nuclear migration at the base of the teleutosorus, whereby this might be effected, but were unable to produce any evidence. Werth and Ludwig (33), however, claim to have seen this migration of nuclei although they also report the presence of binucleate cells in the vegetative mycelium. A recent paper on short cycled rusts by Walker (34), records a variety of ways in which the initiation of the diplophase is effected.

There thus appear to be three main problems requiring further investigation:

- (1) the occurrence of homothallism,
- (2) the origin of the diplophase,
- (3) the problem of hibernation.

MATERIAL AND METHODS

Source of material.

The investigation of the life history of a rust is hampered considerably by the impossibility of growing the fungus independently of the host. The only method for a detailed study is therefore the inoculation of plants day by day, followed by fixation at intervals and examination of the stages of definite age so obtained. Further, Craigie's work on *Puccinia Helianthi* and *P. graminis* suggests that a different development may ensue according to whether these inoculations are the result of monosporidial or of multisporidial infection. Consequently inoculations both with one and with a number of sporidia should be made. In the present study both these methods have been used to elucidate the life-history of *Puccinia Malvacearum*.

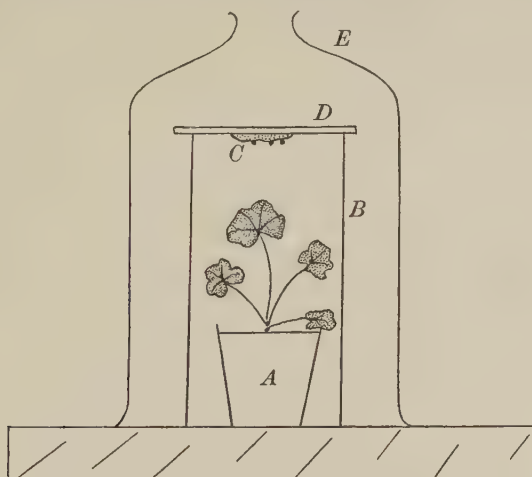
The diseased plants which were used as a source of *Puccinia*

Malvacearum for the inoculation experiments were of the following species: *Althaea rosea*, *A. officinalis*, *Malva borealis*, *M. rotundifolia* and *M. sylvestris*.

The hosts selected for the inoculation experiments were *Althaea rosea*, *A. officinalis* and *Malva borealis*. The seed from which these plants were grown was not specially sterilised, but freedom from the disease was evident as infection never occurred without inoculation. The plants when used were about six months old, and were kept in a special greenhouse. This was not heated and the same range of temperature was experienced in the house as in the field.

Inoculation.

(a) *By many sporidia.* From field observations it is evident that the presence of moisture is one of the essential conditions for infection,



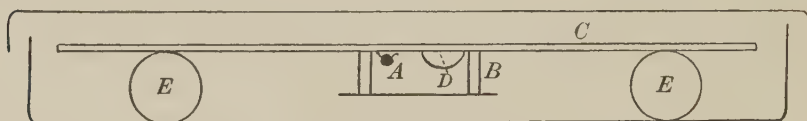
Text-fig. 1. Inoculation apparatus. A, plant; B, cardboard cylinder; C, infected leaf attached with vaseline; D, glass plate; E, bell-jar.

since the disease is most prevalent during periods of damp weather. This fact was borne in mind when making the inoculation experiments in the greenhouse. The plant to be inoculated was therefore first sprayed with tap water and then placed in a damp chamber. At first a bell-jar was considered sufficient, but it was found that moisture was not retained for a sufficiently long period. The use of an inner inoculation chamber was then tried. The form selected was that of a cylinder, the roof of which could be formed by a glass plate bearing the source of infection. Several materials were tried and finally the most successful inoculation chamber was made by wrapping a length of corrugated cardboard round the plant pot to form

a cylinder. The cardboard was especially useful as it retained water, when it was saturated, for a considerable period, and also the size of the chamber could be easily regulated according to the size of the plant pot used. The form of the apparatus is seen in Text-fig. 1. The height of the chamber was always so arranged that the source of infection was four or five inches away from the plant to be inoculated in order to ensure a fairly thin scattering of sporidia. The whole apparatus was then enclosed in the bell-jar to prevent too great evaporation.

To obtain the earliest stages of infection for examination the diseased leaf was placed nearer to the plant to ensure a denser scattering of the sporidia so that less leaf surface had to be examined for these stages.

Preliminary experiments showed that germination of the teleuto-spores began in three hours and continued in any one sorus for a day



Text-fig. 2. Coverslip culture for monospore inoculations. *A*, portion of leaf with fungus; *B*, glass ring; *C*, slide; *D*, drop of water; *E-E*, glass rods.

or more. The plant was therefore left in this moist chamber on the greenhouse bench for forty-eight hours before observations began.

More than a hundred inoculations in this manner have been made day by day during the months October 1930 to June 1931, except for the University vacations at Xmas and Easter. This period includes the winter months and it has therefore been possible to compare the behaviour of the fungus in the greenhouse during this time with events out of doors. On some days in January and February the minimum temperature of the house was as low as 31° F., whilst the maximum for the same day was not more than 40° F., showing that the temperature was little different from that out of doors and yet infection was obtained. Absence of germination out of doors is not therefore due to low temperatures, but rather to unsuitable moisture conditions.

(b) *Monosporidial inoculation.* In making monosporidial inoculations the procedure was as follows (see Text-fig. 2). A small leaf fragment bearing a teleutosorus was fixed by means of vaseline on to the base of a moist chamber formed by a glass ring (diameter half inch) mounted on a slide. A drop of water was placed near the sorus to ensure a moist atmosphere. The chamber was closed by a coverslip coated with a film of plain agar. The whole was inverted and supported by two glass rods in a Petri dish. The apparatus is similar to

that used by Robinson⁽²⁹⁾ in his experiments on the germination of the sporidia of this fungus. The outer chamber formed by the Petri dish was found useful in preventing evaporation.

After two or three hours the culture was examined. During this time the teleutospores had germinated and sporidia were being abstricted. These fell on the agar film and could be seen on examination under the low powers of the microscope. This first scattering was always discarded. A second coverslip was substituted for the first and the culture re-inverted for a period of ten to fifteen minutes. This ensured a fresh scattering of sporidia of known age. These were used for infection experiments.

Isolation of these sporidia was effected by means of a Chambers's micro-manipulator fitted with a micro-pipette coarser than those used for micro-injection as described by Chambers⁽¹²⁾.

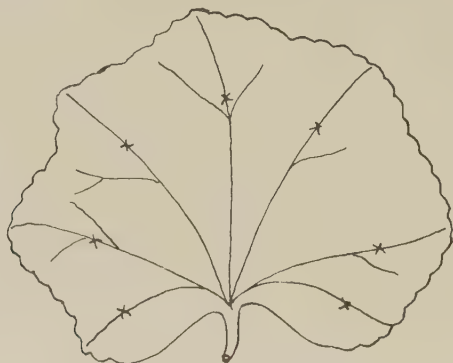
Preliminary experiments had to be made with each pipette to ensure successful isolation of the sporidia. The needle was clamped into position in the manipulator and adjusted for focussing. A film was placed in position above the chamber and the scattering of sporidia brought into focus. Adjustments were then made according to the height of each chamber which varied with the depth of the agar film, and a particular sporidium well isolated from the rest was selected. Slowly the mouth of the pipette was adjusted until the sporidium in its drop of water, with which it is abstricted from the sterigma, was in the centre. The vertical lever was then moved until the pipette was in contact with the agar film and then quickly taken out of contact. Generally the sporidium had vanished. Was it within the pipette or had it adhered to the side? To determine this the pipette was removed from the clamp of the manipulator, the liquid taken up and blown on to a slide. If the sporidium had been sucked up it was ejected from the pipette with the drop of water. The preliminary experiments showed that this was usually the case. Later the ejection of the sporidium was facilitated by first allowing the tip of the pipette to come in contact with, and suck up, a further drop of water.

It was thus apparent that sporidia could be successfully isolated by this means, and it was found that they would germinate if kept in a moist chamber. These preliminary experiments were repeated with each new pipette.

The next step was to apply this method to plant inoculations. The plants to be inoculated were first sprayed with tap water and covered with a bell-jar for four to six hours. At first this was thought to be long enough to produce the required moist and receptive condition of the host, but subsequent experiments showed that better results were obtained by keeping the host plant under the bell-jar for twenty-four to forty-eight hours. A film scattering of sporidia was

then placed over the moist chamber of the manipulator. The first few sporidia isolated and the last few were transferred to a moist chamber (Text-fig. 2) and kept as controls. The middle isolations were used as inoculations, each sporidium being placed on the under side of the leaf as near as possible on to a vein (Text-fig. 3 and Pl. VIII, fig. 1). This fixed the position for further observation.

Between each isolation the pipette was thoroughly washed out in water. That the sporidium had been deposited on the leaf could be demonstrated by re-isolating it from the surface of the leaf and depositing on a microscope slide where it could be identified. This was done at intervals. The bell-jar was replaced over the plant after each monospore inoculation.



Text-fig. 3. Leaf showing points of inoculation of main veins.

After each plant had been inoculated it was placed in an inner chamber for a further forty-eight hours, exactly as for the cultures described above.

One plant every day was set aside as a control. No control plant ever became infected.

Methods of examination.

The first stages of infection were most easily studied by stripping off the upper epidermis of the leaf, fixing it in 70 per cent. alcohol, and staining either with cotton blue in dilute glycerine, erythrosin, or Guéguen's reagent (a mixture of cotton blue and Sudan III in glycerine).

For examination of later stages of development three methods were found useful. The first is that of Mangin (23). Fresh material was boiled for a few minutes in methylated spirit to remove air and then placed in hydrochloric acid, of ordinary strength or diluted by an equal volume of water, to which were added crystals of potassium chlorate (about 1 gm. to 20 c.c. liquid). The tissue was left in this solution until it became bleached, more potassium chlorate being

added to complete the operation if necessary. The material was then washed in water and preserved in alcohol. Tissues thus treated can be successfully stained with cotton blue in dilute glycerine. The method is useful for determining the stage of development and limits of the pustule.

The second method is an adaptation of one used by Wager for the investigation of hyphal branching in agarics. Moderately thin hand sections of a freshly infected leaf were fixed in acetic acid for a few minutes. After washing in water they were stained a deep brown with 5 per cent. Delafield's haematoxylin, and then rinsed in water. After treatment for a few minutes in 1 per cent. potassium hydroxide the preparations were mounted, either in dilute glycerine or glycerine jelly and the fungus separated from the host tissue by tapping the coverslip. This made it easy to observe the distribution of the mycelium and the exact stage of development. It was thus of value in determining the state of material before more careful fixation.

Thirdly, microtome sections were cut from material embedded in paraffin wax. The most useful fixatives were found to be Allen's modification of Bouin and medium-chrom-acetic. Sections were cut 8μ – 15μ in thickness. Haidenhain's iron-alum-haematoxylin, with Congo red as a counter stain as recommended by Blackman(5), Gram's iodine gentian violet, and Flemming's triple stain gave satisfactory results.

HOMOTHALLISM

The short cycled life-history of *Puccinia Malvacearum*, and the absence of pycnidia and aecidia suggest its homothallic nature. By means of the precise method of inoculation described above it has been definitely proved that this rust is homothallic, *i.e.* infection follows from the germination of a single sporidium and teleutospores are formed just as rapidly as they are in nature from mass inoculation. During the course of this work some thousand single sporidium cultures have been made. Of these eighteen have been successful. This may seem slight evidence on which to base the assumption of homothallism, but there are two points to be remembered; firstly, the delicacy of the sporidium itself, and secondly, the amount of handling to which it is subjected during the operation. But all of the precautions taken seemed essential to ensure definitely the isolation of the single sporidium. Moreover, the results represent the whole of the experiments made and naturally the first were less successful than the last. At the end of the work manipulation was so precise that nine out of twenty isolated sporidia caused infection on one plant*. On

* The greater success of these later experiments might also be partly attributed to the fact that they were made in the early spring and therefore the fungus might be expected to be in a more virulent condition. On the other hand, the host plants would be more vigorous and less susceptible to the disease.

the shoot shown in Pl. VIII, fig. 1, every one of the six sporidia put on one leaf germinated and produced teleutosori. The other leaves were not inoculated. The regular distribution of the sori on the leaf is due to the fact that these points on the main veins were always selected for inoculation. The occurrence of homothallism is further supported by the following facts. Firstly, there was never evidence of delayed development such as the sterile aecidia described by Allen⁽¹⁾ in monosporidial cultures of *Puccinia graminis*. Secondly, no more time elapsed between inoculation and the production of the teleutospores in single sporidium cultures than in the mass inoculations. In *Puccinia Helianthi* Craigie^(14, 15) found that although heterothallism was the rule, some monosporidial cultures became spontaneously diploid, but development took longer.

It is hoped to use this technique for the investigation of a long cycled rust, and to demonstrate heterothallism and if possible the distribution of the (+) and (−) sporidia on the promycelium. This is possible in the following way. Teleutospores are first isolated and placed on the agar film on a coverslip at the base of a moist chamber. Their germination can be watched under the microscope. When the sporidia are abstricted the path of their ejection is noted and their final position on the agar film. This coverslip film is then removed from the moist chamber and inverted over the moist chamber of the micromanipulator. The sporidia are then transferred to the leaf in different combinations, according to their position on the promycelium. In this way the distribution of the (+) and (−) sporidia on the promycelium can be demonstrated.

The significance of homothallism in the life-history will be more suitably dealt with in the discussion on the origin of the diplophase.

LIFE CYCLE

Period of development. The length of time required for the development of ripe teleutospores in *Puccinia Malvacearum* was found to differ considerably according to the time of the year at which the inoculation was made. The first visible sign of infection is a tiny white spot or pustule on the under surface of the leaf, which is found on examination to indicate the grouping of the hyphae under the lower epidermis. The change of these spots to a yellow tint marks the change to the diplophase. The following table has been compiled from evidence gathered by fixation and observation of material at known times.

Similar results to these just recorded were obtained from observations of monospore inoculations, as may be seen from the second table.

Clearly there is a marked variation of the rate of development with the temperature, and this again varies with seasonal change. The period of development in these inoculation experiments is shorter in the spring and autumn than in the winter. In the field, infection is

Table I. *Period of development in mass inoculations*

Date of inoculation	First appearance of pustules (days after inoculation)	Initiation of the diplophase (days after inoculation)	Teleuto-spores ripe (days after inoculation)	Period of observation	Average maximum temp. in this period (° F.)	Average minimum temp. in this period (° F.)	Mean temp. (° F.)
*Oct. 28– Nov. 3 Nov. 30	10 18	13 > 18	18 —	Oct. 28– Nov. 21 Nov. 30– > Dec. 18	— — 50	— — 40	— — 45
*Jan. 24– Feb. 3	15	< 20	22	Jan. 24– Feb. 25	65·5	31·5	48·5
Feb. 3	15	< 20	22	Feb. 3–25	62·5	31·5	51·5
*Feb. 17– 18	16	19	22	Feb. 17– Mch. 11	60·5	38	49
May 16	8	10	12	May 16–28	74	51	62·5
June 19	6	8	10	June 19–29	88	57·5	72·5

NOTE. The dates marked with asterisks are the periods of inoculations made on consecutive days and later fixed all on the same day. Thus stages of different age were obtained. The other dates are the days of inoculation, fixation being effected later on consecutive days. This gave the sequence of events in the same mass inoculation.

Table II. *Period of development in monospore inoculations*

Date of inoculation	First appearance of pustules (days after inoculation)	Initiation of the diplophase (days after inoculation)	Teleuto-spores ripe (days after inoculation)	Period of observation	Average maximum temp. (° F.)	Average minimum temp. (° F.)	Mean temp. (° F.)
Feb. 23	14	< 20	23	Feb. 23– Mch. 18	70·5	38·5	54·5
Apl. 19	10	12	18	Apl. 19– May 7	72	44	58
Apl. 20	10	12	18	Apl. 20– May 8	72	44	58

found to be most severe at the former periods, and its severity may partly be explained by this quickening of the life cycle, consequent on the higher temperatures experienced in these months. In the winter and summer there is a decrease in the virulence of the disease. The decrease in the former period is considered in another section of the paper, under hibernation. The summer decrease requires a further explanation. It may be due to lack of moisture or to an increase of temperature above the optimum temperature suitable for development.

At the beginning of the work it was thought that there might be some difference in the rate of development of the rust on different hosts. An experiment was made to determine this. From February 15th until March 4th, a plant of *Althaea rosea*, and one of *A. officinalis* were each inoculated every day. These hosts were selected as the leaves show a marked difference of texture, those of the first being

thick with a thick cuticle, while those of the other are much thinner with little or no cuticle. In spite of this considerable difference, the pustules appeared on the same day on each corresponding pair of plants, a period varying from sixteen days at the beginning of the experiment to thirteen days at the end. Further development also took place at the same time in each. Observation of the behaviour of the fungus on other hosts supports this conclusion.

From these considerations it is apparent that the rate of development of *Puccinia Malvacearum* is independent of the host, but varies appreciably with the temperature.

Entrance of the mycelium and its further development in haplophase

An examination of a one-day old inoculation shows sporidia scattered on the surface of the epidermis. Some have just started to germinate by putting out a beak-like protuberance from the side as figured (Text-fig. 4), or from the hilum. No record has been obtained of more than one germ tube emerging from a single sporidium, although in *Puccinia graminis* Waterhouse⁽³²⁾ reports the presence of two or even three germ tubes. Under abnormal conditions the germ tube of the sporidia of *Puccinia Malvacearum* may branch irregularly. Robinson⁽²⁹⁾ found this to occur when pieces of *Primula* or *Pelargonium* leaf were placed near the sporidia. The age of the sporidia in a one-day old inoculation varies from four to twenty-four hours, and those which were abstricted first are seen to have effected a successful entry, whilst the younger ones have not started to germinate.

Sporidia caught on a dry coverslip enclosing a moist chamber germinate rapidly. Robinson⁽²⁹⁾ investigated the germination of these sporidia, and found that the germ tubes always grew out of a water drop into the moist atmosphere around. They were also found to be negatively phototropic, and although fragments of Hollyhock or Mallow leaf excited no chemotropism the presence of *Pelargonium* or *Primula* leaves inhibited germination. When grown in a coverslip culture in a moist atmosphere development only proceeds for a short time. The sporidium is at first uninucleate, but a nuclear division may occur immediately it is formed so that even before abstriction the sporidium is binucleate (Text-fig. 4). On germination a fairly thin hypha is first put out. The contents of the sporidium slowly pass into this tube. If the germ tube is formed from the hilum, the opposite end of the spore becomes empty, but the nuclei remain almost at the surface of the retreating protoplasm and do not pass into the tube. If germination is lateral, two large vacuoles are formed and the nuclei remain in the central band of cytoplasm. The events just described are the more usual. In some coverslip cultures, however, development of a fine penetration hypha occurs (Text-fig. 5). Just at the tip of the germ tube a very fine hypha (*p*) can be seen coming out at right

angles. In relatively fewer cases still, this penetration hypha swells out again into an infection vesicle (*v*), similar to that formed within the epidermis of the host plant. It was impossible to determine what were the particular conditions which controlled this further develop-



Text-fig. 4. Sporidia germinating on a coverslip.



Text-fig. 5. Sporidia putting out infection vesicles.

ment. The penetration hypha is always put out on the side in contact with the coverslip so that there may be a contact stimulus at work.

Infection of the epidermis of the host plant proceeds as follows. The beak-like protuberance grows a little in length and from the tip of this a fine penetration hypha is put out, just as in the coverslip cultures. The penetration hypha pierces the epidermal wall and

slowly works itself in. Once the cell is entered the contents of the sporidium pass through this fine penetration hypha, to form an infection vesicle. In preparations stained with cotton blue, this perforation can easily be seen (Pl. VIII, figs. 3 and 4).

Eriksson (19) describes the entry of the germ tube of the sporidium into the epidermal cell thus: "wahrscheinlich infolge einer auflösenden Einwirkung des Körpers selbst auf die Epidermiswand bildet sich an dem Kontaktpunktchen ein sehr feines kaum sichtbares Loch durch das sich der Inhalt des Körpers hineinergiesst."

Describing the entry of the germ tube of the sporidium in *Puccinia graminis* Allen (1) reports a thickening of the cell wall of the host at the point of perforation, but this has not been seen in infection by *Puccinia Malvacearum*.

A later stage of infection is shown in Pl. VIII, fig. 2. This is still within the first twenty-four hours after inoculation. Entry has been effected through the narrow neck, the infection vesicle is just forming, and the contents are passing in. The sporidium is lying on the surface of the epidermis, but the vesicle is inside the cell, and is not clearly defined at this focus. The host nucleus is more centrally placed in the cell, and so is at a still different focus. The position is indicated in the drawing. Not all the sporidia which germinate gain entry as is evidenced by the presence of these early stages in older infections.

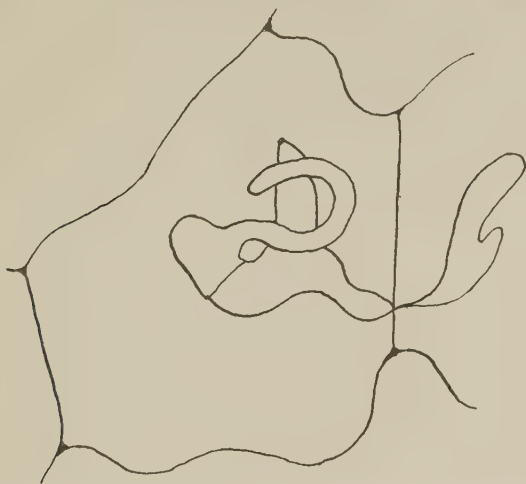
Infection never occurs through a stoma although one may be near at hand. Sometimes a considerable depth of cell wall is penetrated before entry into the cell is effected as shown in Pl. VIII, fig. 3. Infection through the lower epidermis is rare.

The primary infection hypha. Once the penetration tube has entered the cell the tip swells to form what will now be termed the primary infection hypha. Two early stages in the development of this are seen in Pl. VIII, fig. 8. The hypha is at first binucleate, but most frequently a septum is formed a short distance from the tip dividing the swollen hypha into two uninucleate cells. The septation must take place without nuclear division. At this stage in *Puccinia graminis* Allen reports the frequent presence of two nuclei in the cell nearer the point of entry. This suggests the division of one of the two nuclei of the aseptate vesicle and the formation of a wall between the daughter nuclei. It was thought probable that this was the case in *Puccinia Malvacearum*, but there is no evidence for this.

The primary infection hypha grows in length, frequently in the direction of the nucleus of the host cell. The full significance of this is difficult to interpret; possibly the nucleus is the centre of the most active metabolism of the cell and as such offers the best nutrition to the invading hypha (Pl. VIII, fig. 6).

The individual cells of the primary infection hypha have well-marked nuclei, but as the cells become older, the protoplasm becomes

more vacuolate. The cells are characteristic in form for they are much wider than those of the mycelium to which they give rise. The primary infection hypha most commonly consists of three or four cells, though five or six are sometimes present; it is not always confined to the one epidermal cell (Text-fig. 6). The figure also shows how the infection of a neighbouring cell has been accomplished. The hypha in the cell to the right is still broad and irregular and appears to be a continuation of the primary infection hypha. It cannot be confused with the haustoria which are later found in the neighbouring cells of the epidermis. There is as marked a difference in the rate of development of the primary infection hypha as in the complete development of the



Text-fig. 6. A primary infection hypha invading a neighbouring cell.

pustule. In May, this three-celled stage formed in twenty-four hours after inoculation, whilst in the winter months it was not until two or three days after inoculation that this stage was reached.

After this, growth takes place rapidly, and during the next few days marked progress is made in the invasion. The cells of the primary infection vesicle send out finer branches, the beginnings of which are shown in Pl. VIII, figs. 5, 8 and 9. Generally the first cell remains unbranched, but the younger ones branch freely. These branches grow in all directions. At first they are confined to the epidermal cells immediately surrounding the point of the primary infection. They pierce the walls of the host cells through fine perforations always becoming constricted there, but swelling out on the far side. This vegetative mycelium becomes both intercellular and intracellular. As infection usually occurs on the upper epidermis of the leaf the palisade parenchyma with its few intercellular spaces allows very

little development of intercellular mycelium. The hyphae generally enter the palisade cells, form haustoria and then pass on. Once free of this compact tissue the mycelium becomes chiefly intercellular, ramifying in the mesophyll, and putting out large haustoria into the cells of all tissues. Each pustule always develops in relation to one or more vascular strands, and haustoria are formed abundantly in the phloem region. Although entry to the vessels is sometimes achieved, little further development occurs in these. Robinson (28) reported a marked diminution in starch in the regions invaded by the fungus.

A good deal of confusion has existed in regard to the haustoria of *Puccinia Malvacearum*. Taubenhau (31) reports them to be infrequent, but other workers, viz. Sappin-Trouffy (30), Eriksson (19) and Robinson (28) reported them to be of universal occurrence, as found in the present study. They are most variable in form and are well described by Sappin-Trouffy: "Les souçoirs sont très variables. Ils peuvent être fourchus, spirales, rameux, utriculaires ou même pelotonnés à l'intérieur des cellules hospitaliers." They can easily be seen in the fresh condition, but are especially well shown in material stained with gentian violet. Each haustorium is uninucleate and commonly becomes associated with the nucleus of the host cell, in the same way as does the primary infection hypha. By means of plasmolysis experiments, Robinson (28) has shown that the cells of the host plant remain alive for a considerable time after the entry of the haustoria, although various changes consequent on the entry of the haustoria can be demonstrated: the chloroplasts become aggregated round the nucleus, lose their colour and disintegrate; and the nucleus moves from a peripheral position towards the centre of the cell, increasing in size and showing a diminution in chromatin content.

At this time the fungus consists of an intercellular vegetative mycelium composed of long uninucleate segments traversing the spaces of the surrounding tissue, and an intracellular growth, with well-developed haustoria, tapping the food supply of the host tissues. Meanwhile the primary infection hypha is dead and lies in the epidermal cell discoloured with the yellow products of disintegration (Pl. IX, fig. 1). The point of entry of the parasite can be seen in the epidermal cell. From this point, hyphae ramify in the palisade cells immediately below the primary infection, and permeate the leaf tissues. Well-marked haustoria are present at the ends of the intercellular hyphae. The septa are often difficult to see in microtome sections, for the cells of the hyphae are very long, and interlacing. The cells are uninucleate and the nuclei possess well-marked nucleoli.

A little later stage than the one figured shows that the mycelium has invaded the lower epidermis and formed haustoria in its cells. As a response to this check in invasion a second development seems

to take place (Pl. IX, fig. 2). The intercellular mycelium begins to branch repeatedly, the branches grow in the direction of the centre of the pustule, *i.e.* in a reverse direction to their previous development. Each branch assumes a broader form than that of the earlier branches of the vegetative mycelium, and becomes club-shaped. A single nucleus lies at the tip of each, and appears larger than the nuclei of the vegetative mycelium. This massing of the mycelium takes place with great rapidity. Material examined one day, with the mycelium invading the lower epidermis, was found to be in this later condition by the next morning. The pustules still appeared white to the naked eye.

Development of diplophase

The change in colour of the pustule from the white tint of its first appearance to a pale yellow tint, marks the initiation of the diplophase. The colour becomes deeper and deeper until finally the epidermis is ruptured, and the rusty brown teleutospores are exposed. Many investigators have attempted to clear up the cytological details of the change from haplophase to diplophase, but the evidence afforded by the literature is conflicting and the position is by no means clear.

Consequently efforts have been made to collect further evidence for the mode of origin of the diplophase in *Puccinia Malvacearum*, and in the process one has been unavoidably impressed by the possibility of different interpretations of the same phenomena.

Take first the evidence of possible nuclear migration. Several instances of this have been seen but only one case of a nucleus apparently migrating into a *second uninucleate cell*. This is figured in Pl. IX, fig. 3. The nucleus in the lower cell is passing through the wall. At a lower focus the nucleus of the upper cell is seen. A convincing case of nuclear migration is figured in Pl. IX, fig. 4 *b*. Judging by the clear space round the nucleus in the upper cell migration is taking place from the upper to the lower one, but the nucleus of the lower cell could not be found. Pl. IX, fig. 4 *a* shows a similar case. At one focus the nucleus appears as two chromatin masses in separate cells. At a lower focus, these two masses coalesce, and appear to pass through a transverse wall.

No case of initiation of the diplophase by the fusion of two contiguous cells has been seen.

In some sori multinucleate cells are of common occurrence (Pl. IX, fig. 5).

Evidence for the origin of the diplophase by nuclear division not accompanied by wall formation is shown in Pl. IX, fig. 6. Here the terminal cell of the club-shaped hypha is binucleate, and the second cell uninucleate. It was not possible to find another nucleus be-

longing to the second cell in the neighbouring sections and it appears that the terminal cell has become binucleate simply by nuclear division. Again, there is no nucleus in the hypha in contact, which seems to suggest that its nucleus has passed into the upper cell, and the diplophase thus initiated by migration. Other points in favour of this mode of initiation of the diplophase are, firstly, its spontaneous appearance: one day the hyphae are all uninucleate, the next binucleate. Secondly, the nuclei of the club-shaped hyphae frequently appear larger than those of the vegetative mycelium as though preparing for division.

Are we at liberty to assume a simpler mode of origin in homothallic rusts from the more complicated nuclear migration or cell fusion in the heterothallic forms and so give support to the theory of the initiation of the diplophase by nuclear division unaccompanied by wall formation.

On the other hand, the cases of nuclear migration must be considered. Their value here is difficult to assess for they appear to occur infrequently. In homothallic Hymenomycetes, hyphal fusions may occur, and indeed they increase in number long after the whole mycelium has become diploid, thus proving the occurrence of spontaneity in the origin of the diplophase and yet the possibility of hyphal fusions.

For the solution of the problem of the mode of origin of the diplophase, in both long and short cycled rusts, more evidence is needed drawn from many species. Until this is available, the problem of the initiation of the diplophase in short cycled rusts at least, cannot be definitely solved. At present one of the weakest spots in its solution is the possibility of a variety of methods. This is probably due to the difficulties of observation, and the different interpretations possible.

The further development of the binucleate cells was not specially followed.

At maturity the teleutospore consists of a two-celled spore, supported by a fairly long stalk, the wall of which is slightly thickened, but not so markedly as that of the spore. One germ pore is present in each cell. In the upper cell this is terminally placed and consists of a break in the wall just enclosed by a thinner cap. Sometimes this cap can be seen pushed aside on germination. The germ pore of the lower cell is laterally placed just to one side of the cross septum dividing the two cells. The teleutospores are now ready for germination whenever favourable conditions intervene.

The return to the haplophase

The teleutospores are ready for germination about three weeks after the inoculation has been made for they do not require a period of rest as do the teleutospores of the majority of the rusts.

The conditions for germination. In practice it was found that the only condition that need be supplied for germination was moisture. This is the experience of horticulturists who find the disease most prevalent in damp rainy seasons. In the field, germination chiefly occurs after a shower of rain or at night when the dew is falling.

Teleutosori kept dry in the greenhouse at 55° F., or even lower, remain ungerminated for weeks or even months. A clear instance of this and of the immediate germination as soon as moisture should be supplied, was given by a badly infected plant. It had been kept in the greenhouse for some weeks and no germination occurred. One day, however, during watering (always directly on to the soil), the edge of the pot accidentally received a good deal of water. One of the infected leaves was overhanging this. About three hours afterwards a line of sori had germinated corresponding to the arc of moisture given by the damp pot, whilst all the others further from the edge of the pot still remained ungerminated.

A high temperature is not necessary for germination. Teleutospores germinated far more successfully on the bench at 15° C. than in the incubator at 22° C. Indeed germination was found to occur out of doors in winter during suitable moist periods.

Germination of teleutospores. Eriksson(18) recognised two biological forms of teleutospores according to their mode of germination. One is the autumn spore formed from August to October on young plants of the first year, and old plants of the second year. These might germinate in either of the two ways, according to the presence of a water film or a saturated atmosphere. The other is the summer spore formed on hibernated plants of the second year, which germinate by end conidia (oidia) from May to July. In the present study conidia have been formed only in relation to a water film, not to differing teleutospores.

Formation of sporidia. A good account of the germination of teleutospores to form sporidia is given by Buller in his *Researches on Fungi*, vol. III. The process can be very conveniently observed by watching the germination of the spores in a vertical section of a leaf taken through a teleutosorus and mounted in a moist chamber on a small block of plain agar or gelatine. Under moist conditions such as those supplied in the inoculation experiments the contents of each cell of the teleutospore, enclosed in the endospore, emerge as a straight stout promycelium which later becomes curved.

Development by conidia or oidia. If the condition for germination is such that a water film is present, a series of events is seen similar to that described by Blackman(3) for *Phragmidium*, *Gymnosporangium* and *Uromyces*. The promycelium grows straight and continues until it reaches the surface of the water film. This type of promycelium is generally much thinner than the other. The contents collect at the

end, nuclear division takes place and septa are formed as before, but the cells so formed instead of putting out a sterigma and expelling sporidia become detached from one another as broadly elliptical bodies, termed end-conidia or oidia.

HIBERNATION

The teleutospores of *Puccinia Malvacearum*, provided that conditions are favourable, are capable of germination immediately they are formed but the problem of overwintering must rest with either the teleutospore or the mycelium, since the sporidia are essentially delicate and unsuited to survive desiccation for more than a few minutes.

There has been a good deal of interest shown in the hibernation of this rust and several methods have been suggested as to how it is brought about. Moreover, as already suggested above the fungus may behave differently under different climatic conditions, and therefore the sequence of events in America and Sweden may be different from those in England where it has not so far been consistently observed. During the present study the behaviour of the fungus has been watched from day to day from March 1930 to June 1931, in an attempt to establish some definite facts concerning the problem of overwintering.

Diary of observations

At the end of March 1930, the Malviows and Hollyhocks in the Malvaceae bed of the College garden were found to be badly infected. From the fresh appearance of the infected shoots it seemed probable that the teleutosori were the result of an early spring infection. The germination of the teleutospores was tested, and according to the moisture conditions present sporidia or conidia were produced. This was well illustrated by the following experiment. Fairly thin hand sections through a ripe pustule were placed on the surface of a plain agar plate in a Petri dish. The surface of the section in contact with the agar lay in a water film whilst the upper surface was in a damp atmosphere. The teleutospores in the water film germinated to give straight promycelia and conidia, whilst those on the upper surface formed curved promycelia and abstricted sporidia.

During the months of April, May and June teleutospores were germinated practically every day, and no difference could be seen in the germination.

In the long vacation two attempts were made to establish diseased plants in a garden near Manchester, but both were unsuccessful, in that the plants first shed the leaves already formed, and then formed new uninfected leaves, and recovered from the disease. The weather at the time of planting was hot, but damp weather followed within three or four days. In this period the spores germinated. As the

leaves were withering, however, there was no suitable tissue left for infection by the sporidia so formed and consequently the fungus was unable to establish itself.

Early in October the observations were continued at Royal Holloway College. These are of the greatest importance, since they record the behaviour of the fungus during the winter months. In the early autumn infection was heavier than during the summer. At this time plants were being inoculated in the greenhouse by means of diseased leaves brought from plants in the garden. Subsequent examination of the leaf surfaces of the plant under inoculation showed dense scatterings of sporidia. Together with these experiments, two sori were germinated every day in coverslip cultures when films of sporidia were obtained as described above.

New infection was still occurring in the bed as evidenced by the presence of young sori. That this infection was caused by sporidia was shown in the following way. Two plants of *Althaea rosea* were taken from the greenhouse and placed overnight amongst the diseased plants in the garden. Next morning they were removed and taken into the greenhouse, where for two days they were enclosed in bell-jars to provide the requisite moist atmosphere. After twelve or thirteen days young sori appeared on the leaves and matured within three weeks. Considerable new infection was found to occur in the bed until January 16th in the following year. The infection by this time, however, was lighter than in the autumn months. After this only occasional new pustules were seen.

Germination still occurred when sori were brought indoors from the garden. In fact although sometimes the leaves were stiff with frost when gathered, germination of the teleutospores occurred within five hours. On January 22nd four leaves on which pustules were just reaching maturity were noted. The next day one of the pustules was removed and when brought indoors the teleutospores germinated; those left in the field did not germinate. By the beginning of February, however, the pustules no longer appeared rusty brown, but were a greyish white indicating that they had given rise to promycelia. When examined the promycelia were seen to have abstricted sporidia.

A characteristic of the disease at this time was the heavy infection of the petioles. Bearing in mind the suggestion of other workers of the presence of a perennating mycelium, it was thought probable that this petiolar infection was the result of such a mycelium in the shoot tips and rudimentary leaves, renewing growth and passing upwards to the leaves. Fixation and examination of the shoot tips, however, gave no grounds for this assumption. In fact although once a ripe pustule was found on a bud scale the infection was seen to be quite local, and the mycelium did not approach the growing point. Badly infected seedlings were fixed in October in an attempt to find

perennating mycelium, as it was thought that at this time the mycelium might be passing down to the shoot apex there to remain dormant for the winter, but without success.

The plants in the field were most severely affected by a high east wind on March 6th and 7th, and as a result, most of the leaves wilted and decayed in the wet weather that followed. Sufficient pustules remained for germination tests throughout March and April. On April 18th only three pustules were visible on some twenty or thirty plants. During the next few weeks occasional sori appeared, and by May 18th there was considerable infection. The petioles and main veins of the leaves seemed most heavily infected and shoot tips were again fixed and examined for perennating mycelium. No trace of such a mycelium was found.

The history of the fungus in the field throughout the year has now been followed, but before considering the evidence derived therefrom it would be well to add certain observations made on the inoculated plants in the greenhouse, as they throw considerable light on the factors determining teleutospore germination.

In October a set of these greenhouse grown plants, as soon as the teleutospores had reached maturity, were removed to another greenhouse where they could be kept at a temperature of 60°–65° F. indefinitely. They were watered carefully without wetting the leaves. Under these conditions the teleutospores did not germinate but remained viable. Tests were made at intervals from October to June and it was found that immediately moisture was supplied to the teleutospores they germinated.

One fact became increasingly evident, that the time required for germination varies with the age of the teleutospore. This germination period can be conveniently divided into two parts, first the time required for the promycelium to emerge and second, the time required for meiosis and the abstriction of sporidia. This latter period was always the same, it was the length of the first period that altered. Teleutospores of all ages were germinated and the period of germination noted.

The following explanation may be tentatively put forward. It is well established that the stimulus for germination is the presence of moisture. It is possible that this enters the teleutospore wall by differential imbibition which causes swelling of the wall and sets up a pressure in the cell. As a result of this the thin cap-like membrane over the germ-pore yields, and the contents of the teleutospore emerge as the promycelium, enclosed in the extending endospore. The time taken for this imbibition will differ according to the age of the teleutospore. If lately matured the spore will possess a higher water content than one which has undergone desiccation for some months. Consequently the first period, the period required for the

absorption of water, will be longer in the older spore and the time needed to complete abstriction of sporidia consequently longer too.

From these considerations the following facts emerge:

- (1) that the summer and autumn spores do not differ biologically,
- (2) that germination depends on the correct moisture conditions,
- (3) that the life cycle is retarded by the less frequent occurrence of conditions favourable for germination,
- (4) that new infections occur during the winter so that all the pustules present in spring are not the result of a late autumn infection,
- (5) that the teleutospores remain viable for months,
- (6) that a perennating mycelium has not been demonstrated.

Discussion of Hibernation

In a rust like *Phragmidium violaceum* the teleutospores resist all efforts to encourage germination during the winter. Apparently they need a long period of time in order to attain maturity, and cannot germinate until the following spring. But for *Puccinia Malvacearum* the winter conditions do not occasion a period of obligate dormancy except by drought; for as soon as moisture is supplied this assumed dormancy is interrupted by germination.

Plowright⁽²⁷⁾ and Taubenhau⁽³¹⁾ accept this overwintering of teleutospores as a method of hibernation for *Puccinia Malvacearum*. Taubenhau kept infected leaves which he had collected in November, both indoors at a low temperature and out of doors, and by testing spores taken from them at intervals from December to April he found that they still remained viable though more and more slowly as time went on. The important difference between this result and that of the present study is that, in the latter, leaves have been gathered from the plant daily and not kept stored, and also the germination of the spores has been watched in the garden.

Dandeno⁽¹⁶⁾, however, thought this overwintering of teleutospores improbable. Dandeno is the only other worker who notes the development of sori during the winter. He reports that plants growing in Ithaca, N.Y. became infected late in November, and by December white spots had appeared on the leaves, which spots appeared yellow during February and finally matured in April. There has been no such extensive retardation of development observed in the present study, for the slowing down of the life cycle seems dependent on the longer period which intervenes before the appearance of favourable conditions.

Plowright⁽²⁷⁾ suggested that *Puccinia Malvacearum* passed the winter as pustules fallen to the ground, but Taubenhau⁽³¹⁾ asserted that there was no evidence for this. Results of the present work are in agreement with this. Healthy plants potted in soil from the diseased bed showed no signs of infection. It seems probable that infection would be more

easily caused by whole leaves falling to the ground rather than single pustules.

The presence of a wintering mycelium in young leaf rudiments is considered by Grove "to be extremely likely." Taubenhause and Dandeno in America report its occurrence. Eriksson in Sweden has found no evidence for this. I found no overwintering mycelium in *Althaea rosea* and *Malva borealis* even in shoots with infected bud-scales.

Eriksson (19) explained the hibernation of the rust by his mycoplasma theory. In England careful experiments by Bailey (2) showed that plants grown from sterile seed under sterile conditions never became infected, thus proving the absence of mycoplasma in the seed. Moreover, experiments have shown that conidia may develop further to form sporidia and not behave as described by Eriksson. As soon as the water film in which they are formed evaporates, they continue their development in the saturated atmosphere. First a tiny projection is put out to form a sterigma and through this the contents of the conidium pass to form a sporidium at the tip of the sterigma (Text-fig. 7). A drop of water is secreted and abstriction occurs in the usual way.



Text-fig. 7. Germination of conidia to give sporidia.

A more feasible method of hibernation is with the seed of diseased plants. Rust pustules are very commonly formed on the flowers or even the carpels. Unless seed from such a source is properly disinfected there is a chance of contamination of the seedlings. Seed grown from infected plants of *Althaea rosea*, *A. officinalis*, *Malva rotundifolia* and *M. borealis* has been sown and in two seedlings each developed one pustule on the first leaf. It is interesting to note in passing that this is evidence against the statement that infection of seedlings less than six months old rarely occurs. Seedlings from self-sown seed in the garden became heavily infected before they were three months old. This method can be included in the overwintering by teleutospores.

There is another suggestion that the embryo itself contains the fungus, but this has been disproved by Bailey's work and also by other experiments on seed sterilisation.

In England at least, therefore, *Puccinia Malvacearum* seems to persist from season to season, because of the power of its teleutospores to retain their viability for considerable periods, and yet germinate quickly whenever favourable conditions may occur.

With a view to substantiating these conclusions, a short questionnaire was sent to some dozen well-known seedsmen who were good enough to reply in some detail. The disease, it appears, is not treated very

seriously, since the majority have discovered that by the removal of diseased leaves it can be readily controlled; many do not consider it necessary either to sterilise the seed or to change the seed bed. This is, in practice, exactly what one would have suggested from theoretical considerations. Unless the sporidia, formed on germination of the teleutospores, fall upon a fresh leaf where they can germinate at once, there is no danger of infection. Teleutospores that have fallen into the soil have little chance of causing infection. The report of an old custom in the North of England has peculiar significance. It was the practice, it appears, to pile a heap of coal ashes over the crowns of the plants through which the new growth made its way free from disease. This would have the effect of pressing down and burying the old leaves. It is true the ashes might absorb moisture, and so cause the teleutospores to germinate; but their sporidia would be abstricted into the soil and could not cause infection of the new growth.

Biological note. Often when material of *Puccinia Malvacearum* was brought in from the garden for the inoculation experiments, a large proportion of the rust pustules were found to have been eaten, and in a large number a slime track could be seen passing precisely from one damaged pustule to the next. Slugs were watched for and found in the act of eating the pustules and progressing from one pustule to another. The slug was identified as *Limax flavus*. It is obvious that there is no question of spore digestion as is required in some of the coprophilous fungi, since the teleutospores germinate so easily without such aid, nor can this phenomenon be compared with the part played by flies in mixing pycnidia, since this rust is homothallic. But it suggests that slugs may be instrumental in spreading the disease by uneaten teleutospores adhering to their mouth parts. One experiment only was made to discover if this were so. A slug that had just finished feeding on a diseased leaf was transferred to an uninfected plant in the greenhouse and kept under suitable conditions for germination of the teleutospores and sporidia. No infection was, however, obtained. These holes in the leaves recall the observations of other workers. The explanation usually offered is that given by Plowright⁽²⁷⁾ that the sori have fallen to the ground. It is possible that slugs are responsible for most of the holes found in diseased leaves.

SUMMARY

1. A method of monosporidial infection for the rust fungi has been perfected with the help of the micromanipulator.
2. By this means *Puccinia Malvacearum* has been proved to be homothallic.
3. The life-history of the rust has been followed day by day both under natural conditions in the garden and under controlled conditions in the greenhouse.

4. The period required for development of both monosporidial and mass inoculations has been found to vary inversely with the temperature.

5. Shortly after formation and sometimes even before abstriction the sporidium becomes binucleate.

6. Entry to the host cell is effected through the epidermis by the formation of a fine penetration hypha at the tip of a short germ tube.

7. The contents of the sporidium pass through this to form an infection vesicle, an aseptate binucleate hypha, and the primary infection hypha.

8. This primary infection hypha is wide, and becomes septate forming three or four uninucleate cells.

9. After septation each of these cells puts out a thinner hypha which is the beginning of the vegetative mycelium. The vegetative mycelium is both intracellular and intercellular with haustoria in all tissues.

10. The development continues until the lower epidermis is reached.

11. A renewed vigorous growth starts from the centre of the mass of vegetative mycelium. Branches are put out, these ramify repeatedly towards the lower surface.

12. These branches are wider than the early vegetative mycelium and form club-shaped end cells which, however, are still uninucleate.

13. The change to the diplophase is now effected and evidence is cited for the occurrence of this both by nuclear migration and nuclear division not accompanied by wall formation.

14. That the diplophase arises more than once in one sorus is evidenced by the irregular distribution of uninucleate and binucleate cells.

15. Each binucleate cell divides into a lower stalk cell, and an upper cell, which by a further division forms the two-celled teleutospore.

16. The epidermis is ruptured and the teleutospores can germinate immediately they are exposed provided moisture is available.

17. In a moist atmosphere germination takes place to give a curved promycelium and sporidia: in a water film the promycelium is straight and abstricts conidia.

18. The conidia may develop further to give sporidia.

19. The return to the haplophase occurs in the promycelium.

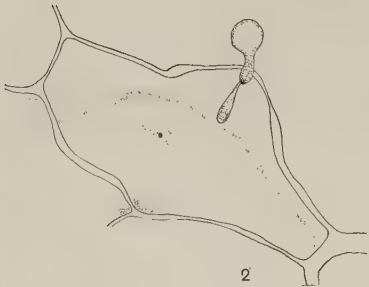
20. There is no biological difference between the teleutospores formed in different seasons.

21. During winter the life cycle is retarded by the less frequent occurrence of conditions favourable for the germination of the teleutospores.

22. New infections occur throughout the winter so that all the



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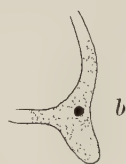
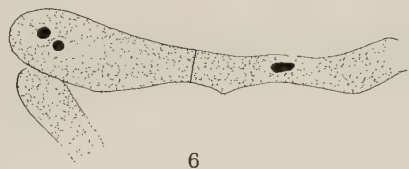
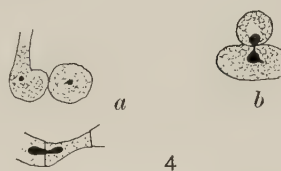
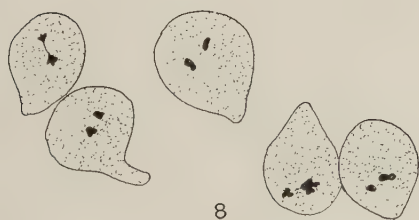
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pustules present in the spring are not the result of a late autumn infection.

In conclusion I should like to thank Miss Blackwell for her helpful advice and encouragement, and the sustained interest she has taken in this work.

DESCRIPTION OF PLATES VIII AND IX

PLATE VIII

- Fig. 1. Photograph of infected plant of *Althea officinalis*. The lower leaf to the right received six inoculations on the veins and shows six pustules.
 Fig. 2. Early stage in infection of upper epidermal cell of *Malva borealis* by a sporidium. The infection vesicle is just forming in the cell.
 Fig. 3. Slightly later stage. The primary infection hypha consists of two uninucleate cells.
 Fig. 4. A three-celled hypha.
 Fig. 5. The two older cells have put out the finer vegetative hyphae.
 Fig. 6. A three-celled hypha showing contact with the host nucleus.
 Fig. 7. A four-celled hypha. The empty sporidium can be seen.
 Fig. 8. Three stages of infection in one host cell. The two youngest show the binucleate vesicle. The oldest is putting out the finer vegetative hyphae.
 Fig. 9. Two similar stages in one host cell. In the right-hand one the nuclei of the primary cells are seen passing up into the finer vegetative hyphae.

PLATE IX

- Fig. 1. A later stage of infection. The mycelium is still vegetative and has not reached the lower epidermis.
 Fig. 2. A still later stage. Infection of the lower epidermis has been effected. The club-shaped hyphae are seen massing towards the lower epidermis.
 Fig. 3. Migration of nucleus into a second uninucleate cell.
 Fig. 4. *a*. Migrating nucleus at two foci. *b*. Another migrating nucleus.
 Fig. 5. Two cases of multinucleate cells.
 Fig. 6. Uninucleate mycelium bearing binucleate club-shaped end cell.
 Fig. 7 *a* and *b*. Initiation of the club-shaped hyphae.
 Fig. 8. Sporidia showing binucleate condition.

LITERATURE CITED

- (1) ALLEN, RUTH T. "A cytological study of heterothallism in *Puccinia graminis*." *Journ. Agric. Res.* XL (1930), 585-614.
 (2) BAILEY, M. A. "*Puccinia Malvacearum* and the mycoplasma theory." *Ann. Bot.* XXXIV (1920), 173-200.
 (3) BLACKMAN, V. H. "On Teleutospore germination." *New Phytol.* II (1903), 10-14.
 (4) — "On the fertilisation, alternation of generations and general cytology of the *Uredineae*." *Ann. Bot.* XVIII (1904), 323-373.
 (5) — "Congo red as a stain for *Uredineae*." *New Phytol.* IV (1905), 173-174.
 (6) BLACKMAN, V. H. and FRASER, H. C. I. "Further studies on the Sexuality of the *Uredineae*." *Ann. Bot.* XX (1906), 35-48.
 (7) BOLLES LEE. *Microtomist's Vade-mecum*. 9th edn. (1928), p. 398.
 (8) BRODIE. "The oidia of *Coprinus lagopus* and their relation with Insects." *Ann. Bot.* XLV (1931), 315-344.
 (9) BULLER, A. H. R. *Researches on Fungi*. III (1924).
 (10) — *Researches on Fungi*. IV (1931), 281.
 (11) — "The Biological Significance of Conjugate Nuclei in *Coprinus lagopus* and other Hymenomycetes." *Nature*, Nov. 1st, 1930, 686-689.
 (12) CHAMBERS, P. "New apparatus and methods for the dissection and injection of living cells." *Journ. Roy. Micro. Soc.* (1922), 373-388.

- (13) CHRISTMAN, A. H. "Sexual Reproduction in the Rusts." *Bot. Gaz.* xxxix (1905), 267-75.
- (14) CRAIGIE, J. H. "Experiments on Sex in Rust Fungi." *Nature*, cxx (1927), 116-117.
- (15) — "Discovery of the Function of the pycnia of the Rust Fungi." *Nature*, cxx (1927), 765-767.
- (16) DANDENO, J. B. "The Life-history of *Puccinia Malvacearum*." *Ann. Rept. Mich. Acad. Sci.* ix (1907), 68-73.
- (17) DUGGAR, *Fungous Diseases of Plants*, 1909.
- (18) ERIKSSON. "Life of *Puccinia Malvacearum* within the host and on the surface." *Phytopath.* xi (1921), 459-463.
- (19) — "Der Malvenrost; seine Verbreitung, Natur, und Entwicklungsgeschichte." *Kungl. Svenska Vetenskapsakademiens Handlingar*, xlvii (1911), No. 2.
- (20) FROMME, F. D. "Morphology and cytology of the aecidium cup." *Bot. Gaz.* lvii (1914), 1-35.
- (21) GROVE, W. B. *British Rust Fungi*. Cambridge University Press (1913).
- (22) HANNA, W. T. "Nuclear association in the Aecium of *Puccinia graminis*." *Nature*, cxxiv (1929), 267.
- (23) MANGIN, L. *Recherches anatomiques sur les Péronosporées*, 1895.
- (24) MASSEE, G. E. *Text-book of Plant Diseases*, 1915.
- (25) MOREAU, F. "Les Phénomènes de la sexualité chez les Urédinées." *Le Botaniste*, xiii (1915), 145-285.
- (26) OLIVE, E. W. "Sexual cell fusions and vegetative nuclear divisions in the rusts." *Ann. Bot.* xxii (1908), 331-60.
- (27) PLOWRIGHT, A. "On the Hollyhock disease." *Gardener's Chronicle*, xviii (1882).
- (28) ROBINSON, W. "On some relations between *Puccinia Malvacearum* and the tissues of its host plant." *Mem. Manchester Lit. and Phil. Soc.* lvii (1913), No. 11.
- (29) — "Some experiments on the effect of external stimuli on the sporidia of *Puccinia Malvacearum*." *Ann. Bot.* xxviii (1914), 331-340.
- (30) SAPPIN-THOUFFY, P. "Recherches histologiques sur la famille des Urédinées." *Le Botaniste*, 5 série (1896), 44-58.
- (31) TAUBENHAUS, J. B. "Morphology and Life-history of *Puccinia Malvacearum*." *Phytopath.* i (1911), 55-62.
- (32) WATERHOUSE, W. L. "Studies in the Physiology of Parasitism. VII. Infection of *Berberis vulgaris* by sporidia of *Puccinia graminis*." *Ann. Bot.* xxxv (1921), 557-564.
- (33) WERTH, E. and LUDWIG, K. "Zur Sporenbildung bei Rost und Brandpilzen." *Ber. d. Deutsch. Bot. Gesell.* xxx (1912), 522-528.
- (34) WALKER, R. I. "Cytological Studies in the Short-Cycled Rusts." *Wisc. Acad. Trans.* xxiii (1927), 565-582.

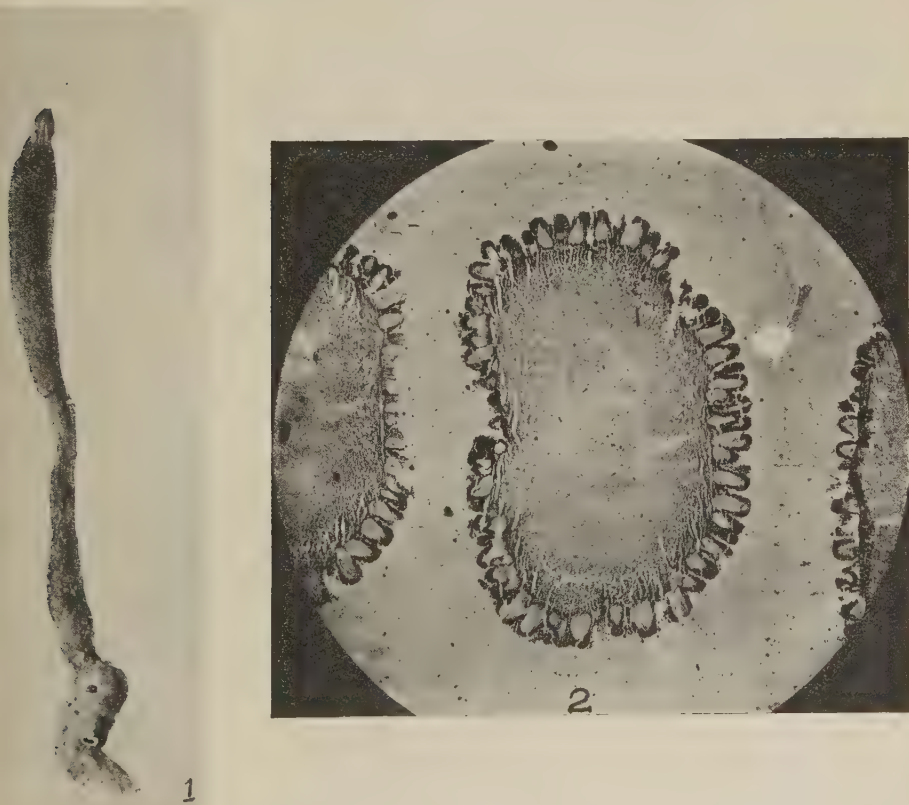
NOTE ON A *CORDYCEPS* FROM TIBET

BY H. CHAUDHURI

(With 3 Text-figures)

IN October 1930, the author received a packet of *Cordyceps* from Professor S. R. Kashyap. A few of the specimens were presented to Professor Kashyap by Lt.-Col. Weir, the Political Officer at Sikkim and others were purchased by him at Yatung, during his last trip to Eastern Tibet.

The specimens were locally known as Yartsa Gungbu—translated literally “in spring grass, in winter worm.” It is a valuable medicine



Text-fig. 1. *Cordyceps sinensis*. Photograph of a Tibetan specimen. Magnified.

Text-fig. 2. Photomicrograph of transverse sections through the perithecial clava.

being used as a tonic by the Tibetans and the Chinese. This is the celebrated Chinese Plant Worm "Hia Tsao Tchong" or "summer grass, winter insect." Professor N. GistGee has given the Chinese history of this *Cordyceps* (*C. sinensis*) in Lloyd's *Mycological Notes* (June 1918, October 1919).

Five complete specimens were received. Text-fig. 1 shows a photograph of a complete specimen. It measures 55 mm. This includes 10 mm. of the underground portion. The barren portion from the ground surface measures 20 mm., fertile portion 18 mm. and the apical sterile portion 2 mm. The perithecial clava reaches a maximum width of 3 mm. The perithecia are superficial and are distributed all round the cylindric head. Text-fig. 2 shows a transverse section photo-



3

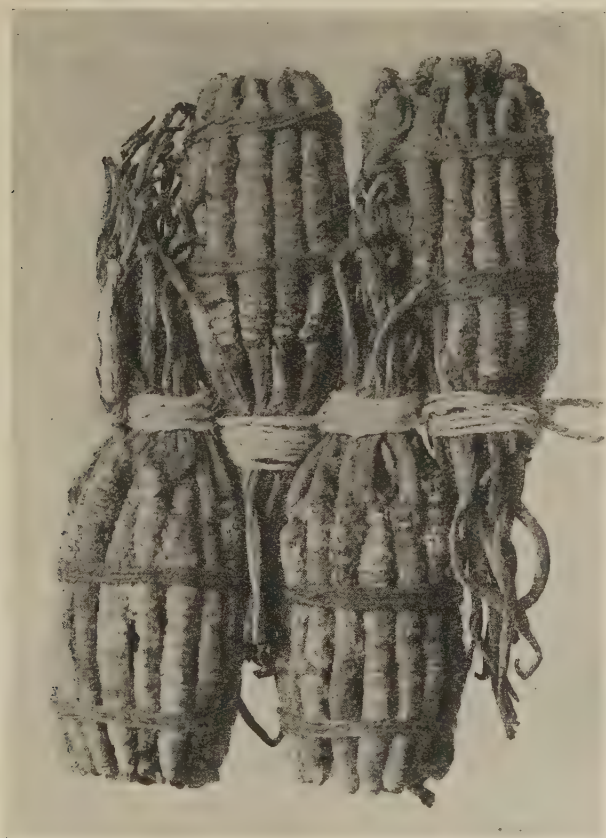
Text-fig. 3. A perithecium, an ascus and an ascospore.

graph of the perithecial clava. The sterile tip is ridged when dried. The perithecia are dark brown when dry. They measure $300\text{--}360\mu \times 150\text{--}180\mu$. They may be loosely adherent to one another at the base but are never immersed in the stroma. The asci are cylindric and measure $300\text{--}350\mu \times 30\text{--}35\mu$. Each ascus contains eight spores. These ascospores are filiform, hyaline and multiseptate and measure $250\text{--}300\mu \times 8\text{--}10\mu$. Text-fig. 3 shows a perithecium, an ascus and an ascospore.

PANJAB UNIVERSITY, LAHORE

May 20th, 1931

A short time before Professor Chaudhuri's paper was received the Rev. J. Walter Sprekley, a missionary from Szechwan, N. China, presented a number of bundles of *Cordyceps sinensis* to the Department of Botany, British Museum. They had been given to him as a peace offering by a Chinese general who apparently thought they were of such value that he was shriven against any activity in future looting.



Four of the bundles as originally tied together are shown in a photograph taken by Mr E. H. Ellis which I have added to Professor Chaudhuri's account.

J. RAMSBOTTOM

REVIEWS

Fungous Diseases of Plants in Agriculture, Horticulture and Forestry. By Dr JAKOB ERIKSSON. 2nd Edition. Translated from the German by WILLIAM GOODWIN, M.Sc., Ph.D. Pp. viii + 526, with 399 illustrations. London: Baillière, Tindall and Cox. 1930.

Professor Eriksson is well known for his investigations on plant diseases, and his "Mycoplasm Theory," though generally discredited by botanists, has stimulated enquiry into the nature of the relationship between host and parasite.

His complete work, *Die Pilzkrankheiten der Kulturgewächse*, consists of two parts. One of these, *Die Pilzkrankheiten der landwirtschaftlichen Kulturgewächse*, was translated into English and published in 1912 under the title of *Fungoid Diseases of Agricultural Plants*. The second part, *Die Pilzkrankheiten der Garten- und Parkgewächse*, which appeared in 1928, has not hitherto been available in English. The two parts have been translated by Dr Goodwin and combined to form one volume under the above title. Its purpose as given in the preface is to present "a general review of the more important fungous diseases which occur in Northern and mid-Europe on field and garden crops and on certain trees of economic or ornamental value."

In covering such a wide field a difficulty is met with in apportioning the text to the diseases according to their merits. This difficulty has not been avoided in Professor Eriksson's book. Compare for instance the description of Hollyhock Rust (7½ pages with 5 illustrations) with that of the Silver Leaf disease (12 lines and no illustrations).

Of the 399 figures many are clear and illustrate well the disease symptoms or the parasites, but some show no detail and are valueless.

Botanists will notice a number of misprints in generic and specific names of hosts and parasites. Other inaccuracies have also crept in. In the description of Plum Rust there is the extraordinary statement (p. 193) that "the uredospores from *Prunus* leaves and the mycelium of the *Anemone* fungus are believed to be perennial in the root stock of the host plants and to germinate in the following year." The description of "Brown Rot of Apples and Pears" is unnecessarily involved, and the assumption that *Sclerotinia fructigena* is the cause of Blossom Wilt of apple trees is untenable.

Some important facts are omitted. Thus there is no mention of the infection of apple twigs by the scab fungus, *Venturia inaequalis*, and of the removal of such infected twigs as an aid in controlling the disease. Bordeaux mixture is the only spray recommended for scab, although lime sulphur has long been used and on some varieties of apples is to be preferred to Bordeaux mixture. In the description of Bunt in wheat there is no reference to the use of copper carbonate dust, although numerous experiments have proved its value.

A few diseases of the infectious virus type are described in a chapter entitled "Diseases not fully investigated." (Has any bacterial or fungal disease of plants been "fully investigated"?) Diseases of this type, as met with in this country, are dismissed in four pages, including a somewhat confused account of "Leaf Roll of Potato," Tomato mosaic, hop mosaic, raspberry mosaic and "reversion" in black currants are not mentioned; these are important diseases in Britain and on the Continent and should have been described if virus diseases are to be included under "Fungous Diseases."

The appendix consists of a "Table of the Fungous Diseases arranged according to the host plants." Since the plants mentioned in this host index number 348 some idea can be gained of the scope of the book. As there is no other recent book in English which covers such a wide field, with special reference to European crops,

this volume will prove a useful book for consultation by all who are interested in plant diseases and parasitic fungi, especially for the numerous references to the more obscure diseases of which the original descriptions are not readily accessible.

H. W.

Dictionary of Biological Equivalents. German-English. By ERNEST ARTSCHWAGER. Pp. 259, 6 plates. London: Baillière, Tindall and Cox. 1930. Price 20s.

It is generally agreed that a knowledge of German is necessary to biologists. Perhaps this is stressed rather more than the necessity for French, because many have to learn it after leaving school. Mycologists possibly have more difficulty with the language than do most other biologists, because of the many ramifications of their subject—for in addition to the technical words peculiar to mycology there are those of allied branches. Everyone who has had to struggle through German papers with the aid of a dictionary has probably compiled a list of technical words which were absent from the dictionary or not given in the compound form required. It is to such that the present dictionary will be found useful, as it gives a comprehensive list of biological terms; it is not likely that as a first edition it is as complete as desirable and it would perhaps be profitable to add the popular names of more plants and animals, for these often prove troublesome. Searching through the book for odd words, a much more critical method than looking through the list of words given, leaves the impression that it will prove very useful to the student for whom it is intended. Six plates give technical terms for shapes of leaves, parts of flowers and anatomical terms. In a further edition it would be useful to extend this to cryptogams.

J. R.

PROCEEDINGS, 1931

MEETING. UNIVERSITY COLLEGE, LONDON. *31st January.*

- A. S. HORNE. Nuclear division in *Spongospora*. Preliminary study of the fungus flora of the air.
- N. M. NITIMARGI. Factors influencing spore formation.
- L. N. SETH. Factors influencing fungal growth.
- W. C. MOORE and A. SMITH. Notes on some interesting fungi recently recorded.
- A. A. PEARSON. A fungus foray in Spain.

MEETING. UNIVERSITY COLLEGE, LONDON. *21st March.*

- D. ASHWORTH. Development of spores in Choanophoraceae.
- M. BRETT. Reversible saltation in *Stemphylium*.
- P. H. GREGORY. The *Fusarium* bulb-rot of *Narcissus*.
- W. M. WARE. Mushroom growing: its scientific and practical aspects.

MEETING. UNIVERSITY COLLEGE, LONDON. *14th November.*

- B. BARNES and R. MELVILLE. Some aquatic fungi new to Britain.
- T. H. HARRISON. A species of *Lambertella* parasitic on apple.
- N. J. G. SMITH. Some of the more curious South African fungi.
- R. G. TOMKINS. Measuring germination.

SPRING FORAY FOR LONDON STUDENTS, BURNHAM BEECHES. *9th May.*

SPRING FORAY, HORSHAM. *29th May-2nd June.*

PHYTOPATHOLOGICAL EXCURSION, ABERYSTWYTH. *1st-3rd July.*

AUTUMN FORAY AND ANNUAL MEETING, BELFAST. *5th-12th September.*

AUTUMN FORAY FOR LONDON STUDENTS, VIRGINIA WATER. *3rd October.*

FORAY WITH ESSEX FIELD CLUB, EPPING FOREST. *10th October.*

FORAY WITH BRITISH ECOLOGICAL SOCIETY, HIGH WYCOMBE.
17th October.

THE BRITISH MYCOLOGICAL SOCIETY was founded in 1896 for "the study of Mycology in all its branches." In its earlier years the Society was carried on much as had been the mycological section of the Woolhope Club, but differed mainly in holding the annual meeting and foray in various parts of the country and in having a journal wholly devoted to Mycology. In the year 1909 an informal spring foray was added to the Society's activities. Since 1921 a much fuller programme has been carried out; the number of forays has been increased while indoor meetings provide for the report and discussion of the results of investigations. Mycologists visiting England are cordially invited to attend any of the meetings of the Society. The programme for the year is issued in January and may be obtained from any of the officers.

The Transactions of the Society appeared annually until 1919 when two numbers were issued; they are now published quarterly. The Transactions deal with every branch of Mycology, and a volume at present contains at least 240 pages with numerous plates and text-figures. Owing to the increase in membership of the Society and the sales of the Transactions, arrangements were made in 1922 for the Cambridge University Press to take over the publication. The annual subscription for Membership of the Society is £1, payable in advance and is due on January 1st of each year. Members are entitled to receive the Transactions of the Society during the year covered by their subscription. For the subscription price to non-members see cover.

Many of the earlier numbers of the Transactions are now out of print, but occasionally copies of these come to hand. Information as to parts available can be obtained on application to the General Secretary.

Papers submitted for publication should be in a condition suitable for press. The Editors are willing to give advice and assistance in the preparation of MSS. Illustrations should be kept down to a minimum. Members are asked to help the Editors to bring the Transactions out regularly. Fifty reprints are provided free of charge: additional reprints may be obtained through the Cambridge University Press.

Attention of members is called to the advisability of sending cultures of fungi to the NATIONAL COLLECTION OF TYPE CULTURES, Lister Institute Chelsea Gardens, S.W. 7, for preservation and distribution.

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